Effect of Ultrasound, Temperature and Pressure Treatments on Enzyme Activity and Quality Indicators of Fruit and Vegetable Juices

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der Technischen Universität Berlin
zur Erlangung des akademischen Grades

Doktor-Ingenieur
-Dr. Ing.-

Genehmigte Dissertation

Promotionsausschuss:

Vorsitzender: Prof. Dr. Dipl. –Ing. U. Stahl
Berichter: Prof. Dr. Dipl. –Ing. D. Knorr
Berichter: Prof. Dr. –Ing. Dr. e.h. F. Meuser

Tag der wissenschaftliche Aussprache: 27 Juni, 2002

Berlin 2002
D 83

Zusammenfassung

Einfluss von Kombinationen aus Ultraschall, Hitze und Druck auf Enzymaktivität und Qualitätsmerkmale von Frucht- und Gemüsesäften

Pektinesterase (PE) ist das hitzestabilste Enzym in Zitrusfrüchten. Dieses Enzym ist verantwortlich für den Pektinabbau, der zum Verlust an natürlicher Trübung im Saft führt. Die Trübung des Saftes ist ein Indikator für die Frische von Säften und eine korrekte Saftproduktion. Die Inaktivierung von PE wird normalerweise als Indikator für eine ausreichende Pasteurisation herangezogen, da dieses Enzym resistenter gegen Hitze ist als die in Zitronensaft vorkommenden Mikroorganismen.

Ultraschallbehandlung ist ein neuartiges Verfahren, das als Alternative zu thermischen Prozessen betrachtet werden kann. Es kann sowohl konvektiven Wärmetransport als auch Kavitation hervorrufen. Dies kann eine Inaktivierung von Mikroorganismen und Enzymen zur Folge haben. Ultraschallbehandlung allein kann die thermoresistente PE auch nach langer Behandlungsdauer nicht inaktivieren. Durch Kombination von Ultraschall und Hitze (TS) kann jedoch die Reduktion dieses Enzyms, abhängig von Behandlungsdauer und Temperatur, geringfügig erhöht werden.

Manothermosonication (MTS) ist eine Methode, bei der eine Kombination von Ultraschall mit Hitze und Druck eingesetzt wird. Diese Kombinationsmethode kann die Inaktivierung von PE bei moderatem Druck (100-300 kPa) deutlich erhöhen. Eine fast vollständige Enzyminaktivierung (94% Inaktivierung bei 70 °C, 300 kPa, 2 min und 96% Inaktivierung bei 80 °C, 200 kPa, 5 min) tritt bei Temperaturen unterhalb von 100 °C auf. Der Erfolg der Enzyminaktivierung ist von pH-Wert, Behandlungsdauer, Temperatur, Druck und Amplitude während der Ultraschallbehandlung abhängig. Ein niedriger pH-Wert des Mediums erhöht die Enzyminaktivierung. Eine PE-Aktivität von 0,55 U/ml wurde bei pH = 2,5, 30 °C und 1 min. beobachtet, während bei pH = 7,5 und sonst gleichen Behandlungsbedingungen die PE-Aktivität 2,5 U/ml betrug. Die Erhöhung der

Behandlungsdauer (um 46 % erhöhte Inaktivierung von 3 min auf 38 min. bei 70 °C, um 65 % erhöhte Inaktivierung von 3 min auf 63 min bei 80 °C) , Temperatur (für 3 min Behandlungsdauer 3,7 % Inaktivierung bei 40 °C, 95 % Inaktivierung bei 80 °C, 98 % Inaktivierung bei 90 °C), Druck (bei 70 °C, 2 min. und 100 kPa lag die PE-Inaktivierung bei 26 %, bei 300 kPa lag sie bei 35 %) und Ultraschall-Leistung (bei 80 °C, 300 kPa wurde die PE um 83,5 % bei 20% Ultraschall-Leistung, um 87% bei 50 % Ultraschall-Leistung und um 91% bei 100% Ultraschall-Leistung reduziert) erhöhte die Enzyminaktivierung ebenfalls. Die Erhöhung der Enzyminaktivierung kann auf drei Mechanismen zurückgeführt werden: Durch Druck erhöhten Ultraschalleffekt (z.B. für MTS Inaktivierung von PE, $D_{60(100KPa)}$ = 3,6 min, $D_{60(300KPa)}$= 1,18 min), durch Ultraschall erhöhte Hitze und Druck (z.B. für die Versuchsreihe der MTS Inaktivierung von PE von frischem Zitronensaft bei 75 °C, 300 kPa, 60% Inaktivierung ohne Ultraschall, 77% Inaktivierung bei 20 % Ultraschall-Leistung, 81% Inaktivierung bei 50 % Ultraschall-Leistung) und durch Temperatur und Druck induzierte chemische Reaktionen.

Der Einfluß von MTS wurde ebenfalls für frischen Zitronensaft und Erdbeersaft untersucht. MTS inaktivierte die PE, während Trübung, Farbe, pH-Wert, Trockensubstanzgehalt und Leitfähigkeit des Saftes unbeeinflusst blieben. Jedoch wurde Ascorbinsäure während der Behandlung und Lagerung abgebaut. Es ist daher wichtig, die optimalen Bedingungen bei der MTS Behandlung (z.B. Entfernung von Sauerstoff vor der Behandlung) zu untersuchen, um die wertgebenden Inhaltsstoffe während der Behandlung möglichst zu erhalten.

Als ein anderes Einsatzgebiet der MTS-Enzyminaktivierung wurde die Inaktivierung von PE bei Tomaten mit moderatem Druck von CO_2-Gas (400 kPa) untersucht. Die dezimale Inaktivierungsdauer (D-Wert) wurde drastisch reduziert. Für Pektinesterase wurde ein D-Wert von 7,39 min bei 60 °C, 400 kPa, 100% Ultraschall -Leistung erzielt, während ohne MTS bei sonst gleichen Bedingungen ein D-Wert von 21 min beobachtet wurde. Derselbe Effekt von kombinierter MTS mit CO_2-Gas wurde im Falle von Polygalacturonase (D_{60} = 12,74 min, $D_{60(MTS)}$ = 5,63 min), Peroxidase (D_{60} = 21 min, $D_{60(MTS)}$ = 7,4 min) und

Polyphenoloxidase (D_{60} = 14,7 min, $D_{60(MTS)}$ = 8,9 min) von Tomaten beobachtet. Diese Inaktivierung war von Temperatur (z.B. PE; D_{60} = 12,8 min, D_{80} = 1 min) und Zeit (z.B. PE Aktivität bei 70 °C, 1min=0,49% U/ml, PE Aktivität bei 70 °C, 5min=0,08 U/min) abhängig. Weitere Untersuchungen sollten sich auf die Aufklärung des Inaktivierungseffektes von kombinierten Verfahren konzentrieren.

Abstract

The most heat stable enzyme in lemon and other citrus juices is pectinesterase (PE). This enzyme induces pectin destabilization, which causes cloud loss in the juice. The cloud presents the fresh-like property and therefore product satisfaction. Inactivation of PE is generally used as an indicator of the adequacy of pasteurization because it is known to be more heat resistant than the common micro-organisms.

Ultrasonic treatment is one of the emerging tools that could be the alternative to thermal processing. It can enhance convective heat transfer as well as generate bubble explosion, which produce local hot spot that can cause micro-organism and enzyme destruction. However, ultrasonication (US) alone cannot inactivate the thermo-stable PE, even at long exposures. The combination of ultrasound and heat (thermosonication, TS) can slightly decrease the activity of this enzyme, which depended on time and temperature.

Manothermosonication (MTS) is a method of combining ultrasonication with thermal and pressure treatment. This method can significantly decrease the activity of PE at the moderate pressure (100-300 kPa) of temperature below 100°C. Almost complete enzyme inactivation (94% inactivation at 70°C, 300 kPa, 2 min and 96% inactivation at 80°C, 200 kPa, 5 min) occurred under the conditions mentioned. The extent of inactivation depended on pH, time of exposure, temperature, pressure and amplitude of the ultrasound. Lowering the pH of the medium increased the inactivation of the enzyme. PE activity of 0.55 unit/ml was obtained at pH 2.5, 30°C, 1 min whereas 2.5 unit/ml was obtained at pH 7.5, 30°C, 1 min. Increase in time of exposure (46% increased inactivation from 3 min to 38 min at 70°C, 65% increased inactivation from 3 min to 63 min at 80°C), temperature (for 3 min treatment time; 3.7% inactivation at 40°C, 95% inactivation at 80°C, 98% inactivation at 90°C), pressure (at 70°C, 2 min PE was 26% inactivation at 100 kPa and 35% inactivation at 300 kPa) and ultrasonic power (at 80°C, 300 kPa, PE was 83.5% inactivation at ultrasonic power of 20%, 87% inactivation at power of 50% and 91% inactivation at power of 100%) enhanced enzyme inactivation. The improvement of the inactivation can be

represented by pressure enhancing US (e.g. for MTS inactivation of PE, $D_{60(100\ kPa)}$ = 3.6 min, $D_{60(300\ kPa)}$ = 1.18 min), US enhancing heat and pressure (e.g. for the experiment of MTS inactivation of PE in fresh lemon juice at 75°C, 300 kPa, 60% inactivation without ultrasound, 77% inactivation at 20% ultrasonic power, 81% inactivation at 50% ultrasonic power) and temperature-pressure treatment inducing chemical reactions.

The decimal reduction times of MTS inactivation of tomato were also dramatically decreased. For pectinesterase, D-value of 7.39 min at 60°C, 400 kPa, 100% ultrasonic power was obtained where D-value of 21 min was obtained at 60°C without MTS treatment. The same phenomenon was observed in polygalacturonase (D_{60} = 12.74 min, $D_{60(MTS)}$ = 5.63 min) peroxidase (D_{60} = 21 min, $D_{60(MTS)}$ = 7.4 min) and polyphenoloxidase (D_{60} = 14.7 min, $D_{60(MTS)}$ = 8.9 min) inactivation. These inactivations depended on temperature (e.g. PE; D_{60} = 12.8 min, D_{80} = 1 min) and time (e.g. PE activity at 70°C, 1 min = 0.49 unit/ml, PE activity at 70°C, 5 min = 0.08 unit/ml). Further investigation should focus on the mechanisms of the combination treatment.

MTS treatment was also investigated on fresh lemon juice and strawberry juice. It has been shown the great potential of this new technology since the MTS treatment could maintain properties such as cloud, colour, pH and conductivity. However, in terms of the nutrition value, ascorbic acid undergoes degradation during the treatment and storage. One needs to investigate further on the optimum treatment of MTS (e.g. oxygen removal) in order to preserve the nutritional indicators in lemon juice.

Publications

Kuldiloke J., Eshtiaghi M. N., Zenker M., Knorr D., Inactivation of Lemon Pectinesterase by Thermosonication, *on publishing in Journal of Food Engineering*.

Kuldiloke J., Eshtiaghi M. N., Zenker M., Knorr D., Inactivation of Lemon Pectinesterase by Manothermosonication; Kinetic Aspect, *on publishing in Journal of Agricultural and Food Chemistry*.

Kuldiloke J., Eshtiaghi M. N., Zenker M., Knorr D., Inactivation of Pectinesterase in Fresh Lemon Juice by Manothermosonication, *on publishing in Lebensmittel-Wissenschaft und –Technologie*.

Kuldiloke J., Eshtiaghi M. N., Knorr D.,Einsatz von Ultraschall kombiniert mit Hitze zur Inaktivierung von Enzymen aus Tomatensaft, *in preparation*.

Acknowledgement

Firstly and foremost, my sincere gratitude goes to Prof. Dr. D. Knorr for his support. He has given me an academic guidance and kept inspiring me throughout my study. He also stood by me every time I faced problems and helped me to keep perspective.

I wish to thank Dr. M. N. Eshtiaghi, who has supported me not only proof-read multiple versions of all chapters of this dissertation, but also provided many suggestions to improve my presentation and clarify my arguments.

My research was necessary to use several laboratory resources. Therefore, I am truly thankful to M. Bunzeit for performing some biological experiments, M. Zenker for controlling ultrasonic equipment and suggesting on my publications, and A. Angersbach for showing me some techniques of Plot-it software programme, which I have used so frequently. My thanks go to Dr. V. Heinz and all people of Food Technology Department, Technical University of Berlin for welcoming and supporting me so warmly to Berlin. I wish to thank W. Tedjo, who was willing to read my work and thus provided some very useful input.

The writing of a dissertation can be a lonely and isolating experience; it is obviously not possible without personal support from numerous people. Thus, I would like to give my special thanks to the Frädrich family for giving me frequent respite and much love, and particularly to P. Euasookkul for her boundless hospitality and consistent encouragement. I wish to thank my friend, S. Muanpawong, for her substantive supports and discussions that pushed me much effort over the past years, and all of my friends and companion in Germany for all helps and supports.

My enormous debt of gratitude to my family for their love, support and unlimited patience can hardly be repaid. My father is not only a sponsor of my study, but he is also a sponsor of my life and career.

Finally, it was a great opportunity to study in Germany. I have had a valuable time and experience that I would never forget. Therefore, I would like to pay my tribute to German study system that provides students extensive facilities, opportunities and knowledge.

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Abbreviations

a	colour tone (red and green)
b	colour tone (yellow and blue)
A	Enzyme activity (unit/ml)
A_i	The initial activity of the enzyme (unit/ml)
A_f	The residual activity of the enzyme (unit/ml)
B	The frequency or Arrhenius factor
C	Constant term, equal to k_BT/h
D	Decimal reduction time (min)
D_T	Decimal reduction time at temperature T (min)
D_{refT}	Decimal reduction time at reference temperature (min)
E_a	Arrhenius activation energy (kJ/mol)
E_I	Inactive enzyme
E_N	Native or active enzyme
E_{N^*}	Transition state enzyme
h	Constant of Planck (6.262×10^{-34} Js)
ΔH	Enthalpy
HP	High pressure treatment
k	Specific rate constant (min^{-1})
k_B	Boltzmann constant (1.38×10^{-23} J/K)
k_{refP}	Inactivation rate constant at reference pressure (min^{-1})
k_{refT}	Inactivation rate constant at reference temperature (min^{-1})
K^*	"Quasi-equilibrium" constant
L	Light value
MS	Manosonication
MTS	Manothermosonication
n	The order of reaction
pD	The monitored flowrate in the pilot plant (l/h)

pS	The monitored pressure in the pilot plant (kPa)
P	Pressure treatment
P	Treatment pressure (kPa)
P_{ref}	Reference pressure (kPa)
PE	Pectinesterase
PG	Polygalacturonase
POD	Peroxidase
PPO	Polyphenoloxidase
PS	Presonication
PTS	Postsonication
R	Gas constant (J/mol K)
t	treatment time (min)
T	Thermal treatment
T	Treatment temperature (°C)
T_{ref}	Reference temperature (°C)
TS	Thermosonication
US	Ultrasonication or ultrasonic amplitude
V_a	Activation volume (cm^3/mol)
z	Temperature dependence (°C)

Part I

Introduction

Chapter 1

General consideration and aims

Commercial lime and lemon juices are among the world most important citrus products. For example, these juices are used as a common ingredient in most of the traditional Asian cooking. Moreover, they are necessary for many global food productions, such as lemonade drink, marmalade, jams, candies, jellies, desserts, pharmaceutical products, and medicines.

Lemon juice, itself, is a colloidal suspension of cellular and polymer particles. This cloudy appearance is an important property of the juices since it gives the natural appeal of the fresh juices. Colloidal stability is maintained by pectin molecules through a complex and not well understood mechanism. Cloud loss of citrus juices is an intensively studied problem in food technology. It is due to the action of endogenous pectinesterase (PE) on pectin substance. PE catalyzes the de-esterification of pectin molecules. De-esterified pectin molecules are able to interact through calcium bridges, leading to cloud loss and phase separation in single-strength lemon juices and gelation in their concentrates. Stabilization of cloud in citrus juices requires the inactivation or inhibition of PE (Vercet, et al., 1999).

Several strategies have been used to inhibit or inactivate PE avoiding the negative effects of intensive heat treatments. Inhibition of PE by polyphenols (Hall, 1966; Pilnik and Voragen, 1991), inhibition by specific proteic PE inhibitors (Castaldo et al., 1991), or inhibition by the oligogalacturonides produced by the action of added polygalacturonase or pectinylase (Baker and Bruemmer, 1972; Krop and Pilnik, 1974; Termote et al., 1977) have been suggested as alternatives to the heat treatments. Other strategies rely on PE

inactivation by nonthermal treatments such as high pressure (Irwe and Olson, 1994; Donsi et al., 1996; Cano et al., 1997; Knorr, 1998), low pH values (Owusu-yaw et al., 1988), or supercritical carbon dioxide (Arreola et al., 1991; Balaban et al., 1991; Ishikawa at al., 1996).

Another possible alternative is ultrasound in combination with heat and pressure (Manothermosonication; MTS). MTS is an emerging technology that efficiently combines the inactivating effect of heat and ultrasonic waves (Burgos, 1998).

MTS has been proved to be an efficient tool to inactivate some other enzymes, such as lipoxygenase, peroxidase, and proteases and lipases from psychrophic bacteria (Lopez at al., 1994; Sala at al., 1995; Vercet at al., 1997).

Most results reported in the scientific literature, in fact, relate deactivating and destructive action of ultrasound only to its frequency and fail to provide information about the dependence of the treatment efficiency on the actual power and power density of ultrasound. In addition, no definite experimental evidence has been reported on the efficiency of ultrasound in batch or in continuous applications.

The aims of the study were to investigate the effect of ultrasound on the inactivation of lemon pectinesterase and juice quality. One further aim was the evaluation of the kinetic parameters of the MTS effect on lemon and tomato pectinesterase. Finally, the potential of the MTS in fruit and vegetable (lemon, strawberry, tomato) juice industry was determined.

Part II

Theory

Chapter 2

Ultrasonic science

2.1 Sound ranges

The range of human hearing is from about 16 Hz to 18 kHz. Ultrasound is the name given to sound waves having frequencies higher than those to which the human ear can respond (i.e. > 18 kHz). The upper limit of ultrasound frequency is one, which is not sharply defined but is usually taken to be 5 MHz in gases and 500 MHz in liquids and solids. The use of ultrasound within this large frequency range may be divided broadly into two areas. The first area involves low amplitude (higher frequency) propagation, which is concerned with the effect of the medium on the wave and is commonly referred to as “low power” or “high frequency ultrasound”. Typically, low amplitude waves are used to measure the velocity and absorption coefficient of the wave in a medium in the 2 to 10 MHz range. It is used in medical scanning, chemical analysis and the study of relaxation phenomena. The second area involves high energy (low frequency) waves known as “power ultrasound” between 20 and 100 kHz which is used for cleaning, plastic welding and, more recently, to effect chemical reactivity.

Ultrasonic waves are generated by mechanical vibrations of frequencies higher than 18 kHz. When these waves propagate into liquid media, alternating compression and expansion cycles are produced. During the expansion cycle, high intensity ultrasonic waves make small bubbles grow in liquid. When they attain a volume at which they can no longer

absorb enough energy, they implode violently. This phenomenon is known as cavitation. During implosion, very high temperatures (approximately 5000 K) and pressures (estimated at 50000 kPa) are reached inside these bubbles (Sala et al., 1998).

2.2 Mechanisms and effects

High-intensity acoustic radiation causes various changes as is propagates through a medium. These changes can be explained by several mechanisms, but not all mechanisms involved are known or well understood. Most of the reported effects and proposed mechanisms can be summarized as follows:

Heating: As a result of specific absorption of acoustic energy by membranes and biomaterials, particularly at their interfaces, a selective temperature increase may take place (Floros and Liang, 1994). This heating effect was assumed to be responsible for the significant increase in diffusion of sodium ions through living frog skin under ultrasound (Lehmann and Krusen, 1954). The increase in permeability of the living membrane was so large that its selectivity was completely lost. Later theoretical and experimental results do not support the early assumptions. Floros and Liang (1994) emphasized about the heat balance equation to calculate loss of ultrasonic energy as it propagates through a medium. They mentioned that the temperature change due to absorption at a solid wall, under given conditions, was 0.1°C for water and about 1°C for air. These results were verified experimentally. Other investigators claim that localized temperature increase of up to 5000 K can be expected for a few nanoseconds in a sound field (Suslick et al., 1985).

Cavitation: Acoustic cavitation is the formation, growth, and violent collapse of small bubbles or voids in liquids as a result of pressure fluctuation (Suslick, 1988). In general, cavitation in liquids may cause fast and complete degassing; initiate various reactions by generating free chemical ions (radicals); accelerate chemical reactions by facilitating the mixing of reactants; enhance polymerization/ depolymerization reactions by temporarily dispersing aggregates or by permanently breaking chemical bonds in polymeric chains; increase emulsification rates; improve diffusion rates; produce highly concentrated

emulsions or uniform dispersions of particles; assist the extraction of substances such as enzymes from animal, plant, yeast, or bacterial cells; remove viruses from infected tissue; and finally, erode and break down susceptible particles, including micro-organisms.

Structural effects: When fluids are placed under high intensity sound fields, the dynamic agitation and shear stresses produced affect their structural properties, particularly their viscosity. Usually, Newtonian fluids maintain their characteristics, but dilatants and thixotropic fluids tend to either stiffen or become less viscous, respectively (Ensminger, 1986).

Compression and rarefaction: When high-intensity acoustic energy travels through a solid medium, the sound wave causes a series of rapid and successive compression and rarefaction, with rates depending on its frequency. In turn, the material is subjected to a rapid series of alternating contractions and expansions, much like when a sponge is squeezed and released repeatedly. This mechanism, known as "rectified diffusion", is very important in acoustic drying and dewatering and noticeable moisture migration takes place overall (Ensminger, 1986). In more dense materials that are practically incompressible, the alternating acoustic stress facilitates dewatering by either maintaining existing channels for water movement or creating new ones. Dense materials usually "fracture" under acoustic stress. Microscopic channels are created in directions normal to wave propagation during rarefaction, or parallel to wave propagation during compression (Floros and Liang, 1994). The same mechanism results in elevated and reduced pressure at gas/liquid interfaces, and therefore increases evaporation rates. Although the pressure variation introduced by the sound wave is very low, its effect is strong because of the rapid rate of pressure oscillation.

Turbulence: High-intensity ultrasound in low-viscosity liquids and gases produces violent agitation, which can be utilized to disperse particles (Ensminger, 1988). At liquid/solid or gas/solid interfaces, acoustic waves cause extreme turbulence known as "acoustic streaming" or "micro streaming" (Nyborg, 1965). This reduces the diffusion

boundary layer, increases the convection mass transfer, and considerably accelerates diffusion in systems where ordinary mixing is not possible.

Other: A number of other effects and mechanisms have been reported. Ultrasonic waves of high intensity assist the cleaning of surfaces. This mechanism has been used to prevent binding or formation of filter cake and enhance filtration rates (Floros and Liang, 1994). Under certain conditions, high-intensity ultrasound causes coalescence of many types of particles and can be used effectively in low-concentration suspensions.

Chapter 3
Effect of ultrasound on enzyme inactivation

3.1 Inactivation kinetics of enzymes

At a constant pH, degradation of nutrients and inactivation of enzymes and micro-organisms are usually described by first-order rate expressions. Temperature effects on these reactions usually agree with the Arrhenius equation. At different pH values these reactions occur via different mechanisms, so the activation energy and frequency factor in the Arrhenius equation vary with pH in a complex manner (Uelgen and Oezilgen, 1993). Response surface methodology is preferred for optimization of such complex processes. In this technique dependent variables (fraction of the surviving enzyme activity, logarithm of the surviving microbial population and fraction of the vitamin retained) are described as arbitrary functions of the independent variables (pH of the juice, processing time and processing temperature). A sufficiently large number of terms are included for these functions to mirror the variations in the experimental data very closely.

3.1.1 Evaluation of *D* and *z* values of enzyme for thermal process calculation

As in thermobacteriology, enzymatic inactivation features kinetic parameters that make it possible to compare the degree of inactivation and the thermal treatment defined by the temperature-time curve. The most important kinetic parameter is the decimal reduction time (D_T) and its dependence on temperature expressed by z. The decimal reduction time was calculated according to Stumbo (1973) by equation:

$$D_T = t/(\log A_i - \log A_f) \qquad (3.1)$$

where A_i is the starting activity; A_f is the residual activity that survives the thermal treatment; and t is the thermal treatment time at temperature T in minutes.

The z parameter was derived from $\log D_T$ values at different treatment time versus temperature. The z parameter indicates how many degrees the temperature must change for the decimal reduction time to be 10 fold higher or lower.

$$z = -(T - T_{ref})/(\log D - \log D_{refT}) \qquad (3.2)$$

where T = Temperature in °C (or K) for the lower temperature
T_{ref} = Temperature in °C (or K) for the higher temperature
D = D-value for the lower temperature (min)
D_{refT} = D-value for the higher temperature (min)

3.1.2 Order of the reaction

Protein and enzyme denaturation often follows first-order kinetics; by definition that a single molecule undergoes a conformational change. However, when more than a single kind of enzyme is present, as is frequently the case in food enzyme preparations, the kinetics may be complex.

If first-order kinetics can be assumed, the process is

$$E_N \xrightarrow{k} E_I \qquad (3.3)$$

where E_N is the native or active enzyme, E_I is the inactive enzyme, and k is the specific rate constant for the inactivation process. Mathematically the decrease in active enzyme becomes

$$-d[E_N]/dt = k[E_N] \quad \text{or} \quad -dA/dt = kA \qquad (3.4)$$

where A is enzyme activity, then integration gives

$$\ln([E_N]/[E_{Ni}]) = -kt \quad \text{or} \quad \ln(A/A_i) = -kt \qquad (3.5)$$

where E_{Ni} is the initial native, active enzyme and A_i is initial enzyme activity.

3.1.3 Temperature dependence

The most commonly used mathematical expression for the effect of temperature on rates of chemical processes, including enzyme reactions, is Arrhenius relationship,

$$k = B\, exp^{-Ea/RT} \qquad (3.6)$$

$$\ln k = \ln B - E_a/RT \qquad (3.7)$$

where B is the frequency or Arrhenius factor and E_a is the Arrhenius activation energy. Equation 3.7 indicates a linear relationship when $\ln k$ is plotted against $1/T$. The slope of this plot is $-E_a/R$. The magnitude of E_a indicates the temperature dependence of the reaction in question.

For a definition of reference temperature, equation 3.17 can be rewritten as

$$\ln k = \ln k_{refT} - \{E_a/RT[(1/T)-(1/T_{ref})]\} \qquad (3.8)$$

where k_{refT} = inactivation rate constant at T_{ref} (min^{-1})

T_{ref} = reference temperature (K)

3.1.4 Transition state theory and pressure dependence

The similarity between the Arrhenius and Van't Hoff equations should be noted. However, E_a is not identical to enthalpy (ΔH). Yet, the similarity between the two equations suggests that values for E_a may provide some insight into the thermodynamic nature of chemical processes. The transition state, or Eyring theory, is the most successful attempt to relate irreversible kinetic information to thermodynamic information. In brief, this theory suggests that native enzyme E_N , in an irreversible reaction, goes through a transition state E_{N*}, which is in equilibrium with the native enzyme.

$$E_N \overset{K*}{\Leftrightarrow} E_{N*} \Rightarrow E_I \qquad (3.9)$$

where $K*$ = "quasi-equilibrium" constant

The $K*$ value does not really represent an equilibrium but rather the probability that reactants will get into the activated state and decompose. The rate of disappearance of E_N is given by

$$-dE_N/dt = C[E_{N*}] \qquad (3.10)$$

where C = constant term, equal to k_BT/h

k_B = Boltzmann constant (1.38×10^{-23} J/K)

h = constant of Planck (6.262×10^{-34} Js)

Since $K* = [E_{N*}]/[E_N]$, eqn. 3.10 becomes

$$-dE_N/dt = k[E_N] \qquad (3.11)$$

where $k = CK^* =$ the specific rate constant for the transition process

From $\Delta G^* = -RTlnK^*$, then k can be derived as follows:

$$k = (k_B T/h)exp(-\Delta G^*/RT) \qquad (3.12)$$

The dependence of the inactivation rate constant on pressure can be given as follows:

$$\frac{\delta ln\, k}{\delta P} = \frac{\delta ln\, K^*}{\delta P} \qquad (3.13)$$

$$-RT\frac{\delta ln\, k}{\delta P} = -RT\frac{\delta ln\, K^*}{\delta P} = \frac{\delta \Delta G^*}{\delta P} = \Delta V^* \qquad (3.14)$$

where ΔV^* is activation volume (cm^3/mol). From this equation, it can be derived that inactivation is enhanced by pressure if the volume decreases. For the reference rate constant at reference pressure, the equation 3.14 can be derived as

$$ln\, k = ln\, k_{refP} - [V_a/RT(P-P_{ref})] \qquad (3.15)$$

where k_{refP} = inactivation rate constant at T_{ref} (min^{-1})

P_{ref} = reference pressure (kPa)

3.2 Application of ultrasound on enzyme inactivation

3.2.1 General information

It has been known for many years that ultrasound can be employed as a method of inhibiting enzyme activity. Peroxidase, which is found in most raw and unblanched fruit and vegetables, is particularly associated with the development of off-flavours and browning pigments. The original activity of peroxidase was progressively reduced by 90% as ultrasound was applied over a 3 h period. Nearly 60 years ago Chambers reported that

pure pepsin was inactivated by sonification probably as a result of cavitation. The implosion of "internal cavitating" bubbles induced by ultrasound waves generates microscopically small hot spots (temperatures estimated at 5000 K) and local pressures of 50000 kPa (Suslick, 1988). In MTS, moderate pressure is applied to allow cavitation at temperatures close to or above the boiling point. This also increases the implosion intensity, which is related to the difference between the static pressure and the vapour pressure inside the bubble (Vercet, 1999).

The introduction of refrigerated tanks for bulk storage of milk before heat treatment has reduced spoilage by mesophilic micro-organisms but favours the growth of psychrotropic bacteria. Although these micro-organisms are easily destroyed under standard heat treatments, many of them produce extracellular lipase and protease, which withstand UHT treatment (Stead, 1986). These thermoresistant enzymes can reduce the quality and shelf-life of heat-treated milk and other dairy products. The simultaneous application of heat and ultrasound waves under pressure (manothermosonication; MTS) has also been found to be more effective than heat treatment in the inactivation of these heat resistant protease and lipase secreted by *P. fluorescens* (Vercet et al., 1997).

3.2.2 Application of ultrasound

3.2.2.1 Ultrasonication

Ultrasonication (US) is operated at low temperature. Therefore, a product with heat-sensible components can be treated. However, the treatment time is actually long during the inactivation of enzymes and/or micro-organisms, which may cause high-energy requirement. Normally, this treatment will need to be combined with other techniques to optimize the process.

3.2.2.2 Presonication

In case of presonication (PS), the product is pretreated by ultrasound before subjected to the heat and/or pressure treatment. In this case, the enzymes and/or micro-organisms are resistant to heat and pressure. Pre-treatment with ultrasound can minimize the resistance of

enzymes and micro-organisms, which can be completely inactivated by the following temperature and pressure treatment.

3.2.2.3 Thermosonication

In thermosonication method (TS), the product is subjected to ultrasound and moderate heat simultaneously. This technique shows the same inactivation level compared to the treatment without ultrasound at high temperature. The temperature, however, is rising during the treatment. Therefore temperature control is required.

3.2.2.4 Postsonication

By applying postsonication (PTS), the product is treated with heat and/or pressure before being subjected to ultrasound. This technique is still not popular and there are no experimental data available on this technique so far.

3.2.2.5 Manosonication

Manosonication (MS) provides the possibility to inactivate enzymes and/or micro-organisms by combining ultrasound with moderate pressure 100 - 300 kPa at low temperature.

3.2.2.6 Manothermosonication

Manothermosonication (MTS) combines the ultrasound with moderate temperature and moderate pressure in order to inactivate enzymes and/or micro-organisms. The ultrasound generates the cavitation or bubble implosion in the media. This implosion can cause inactivation of enzyme and destruction of micro-organisms. The simultaneous pressure treatment maximizes the intensity of the explosion, which increases the level of inactivation.

Chapter 4
Application of ultrasound in food industry

4.1 General information about ultrasound in food industry

There is presently much industrial interest in developing mild food preservation procedures, which could replace the severe heat-based methods, which are currently in common use. Often termed minimal processing, the benefits of these approaches are an important aspect of current and future commercial product development. Quality attributes, which can be protected by application of minimal process technologies, are: flavour and odour, visual appearance, i.e. colour and texture, nutrition value, absence of additives. Minimal processing can be applied to a wide variety of foods including short shelf-life products such as fresh fruit and vegetables, chilled ingredients and convenience dishes through to long-life ambient stable foods such as cooked meats and vegetables. This commercial challenge has opened up new opportunities for combined preservation systems incorporating mild heat treatments or low food additives and provided impetus for the new variables for microbial control.

The use of ultrasound within the food industry has been a subject of research and development for many years and, as is the case in other areas, the sound ranges employed can be divided basically into high frequency, low energy, **diagnostic ultrasound** in the

MHz range and low frequency, high energy, **power ultrasound** in the kHz range. Application of ultrasound in food processing can be classified into two main categories:

1. monitor a process or product
2. affect a process or product

4.1.1 Diagnostic ultrasound

Up to a few years ago the majority of applications and developments involved non-invasive analysis with particular reference to food quality assessment, e.g. by monitoring the attenuation of an ultrasound pulse it has been proven possible to determine the degree of homogenization of fat within milk. Several reports have summarized, classified, and critically reviewed the present and future outlook of low-intensity, high frequency ultrasound for process/product monitoring (Floros and Liang, 1994). Useful industrial applications include texture, viscosity, and concentration measurements of many solid or liquid foods; composition determination of eggs, meats, fruits and vegetables, dairy, and other products; thickness, flow, and temperature measurements for monitoring and control of several processes; and non-destructive inspection of egg shells and food packages.

4.1.2 Power ultrasound

Application of ultrasound to directly improve processes and products is less popular in food manufacturing, but well recognized in other industries. High-intensity sound is mainly used for such applications with frequency either in the sonic (< 18 kHz) or ultrasonic (≥ 18 kHz) range, depending on the application. The beneficial use of sound is realized through its chemical, mechanical, or physical effects on the process or product. In fact, a new branch of chemistry called sonochemistry has been created to take advantage of the chemical effects of ultrasound (Suslick, 1986).

4.1.2.1 Acceleration of the reaction

General applications include acceleration of conventional and decomposition reactions, degradation of polymers, and polymerization reactions (Floros and Liang, 1994). When particles of material in a liquid suspension are subjected to sonication a number of physical and mechanical effects can result. Large particles are subject to surface erosion (via cavitation collapse in the surrounding liquid) or particle size reduction (due to fission through interparticle collision or the collapse of cavitation bubbles formed on the surface). Recent studies on the effect of sonication on suspended powders have shown that the particles can be forced into violent collision that, in the case of metals, fusion can occur. In some cases the colloding powders undergo chemical reaction. Thus when copper and sulphur are sonicated together in hexane for 1 hour, 65% Cu_2S is generated (Goh et al., 1994).

4.1.2.2 Cleaning and degassing liquids

The mechanical and physical effects of sound are utilized to improve cleaning of surfaces (Crawford, 1963). The cavitational effects, which are the basis of sonochemistry, are also the reason for the extremely effective uses of ultrasound for the degassing of liquids. Any dissolved gases or gas bubbles in the medium act as nuclei for the formation of cavitation bubbles. Such bubbles are not easily collapsed in the compression cycle of the wave due to the fact that they contain gas and they will continue to grow on further rarefaction cycles, filling with more gas and eventually floating to the surface. Since the rarefaction cycles are taking place extremely rapidly (around 40,000 times per second using an ultrasonic bath) the bubbles grow so quickly that degassing appears to occur almost instantaneously.

4.1.2.3 Crystallization

Power ultrasound has proved to be extremely useful in crystallization processes (Mason et al., 1996). It serves a number of roles in the initiation of seeding and subsequent crystal formation and growth. It also has a secondary property, which is beneficial in such

processing applications, namely that the cleaning action of the cavitation effectively and thereby ensures continuous efficient heat transfer. It has been reported that ultrasound can be used to clarify wines through the precipitation of potassium bi-tartrate (Mason et al., 1996). The texture of food products will be affected by the size of undissolved sugar crystals dispersed in the material. Size will also affect the rate of dissolution of sugar in food preparation. For these reasons the control of sugar crystallite size is important. Normal crystallization of sugar from concentrated sucrose solutions leads to large uneven sized crystals, which can be broken down by subsequent sonication (Mason et al., 1996). One very important area related to crystallization in the food industry is the formation of ice crystals during freezing of water.

4.1.2.4 Drying and filtration

The requirement to remove suspensions of solids from liquids is common to many industries including chemical, engineering as well as food. This separation can be either for the production of solid-free liquid or to isolate the solid from its mother liquors. Ultrasonic filtration of particulate matter from a liquid is now arousing some interest since the rate of flow through a filter can be increased substantially on application of ultrasound. There are two specific effects of ultrasonic irradiation which can be harnessed to improve the filtration technique: (1) sonication will cause agglomeration of fine particles and lead to rapid filtration, which (2) will supply sufficient vibrational energy to the system to keep the particles partly suspended and therefore leave more free “channels” for solvent elution. The combined influence of these effects has been successfully employed to enhance vacuum filtration of industrial mixtures such as coal slurry, which is a particularly time consuming and difficult process (Senapati, 1991).

4.1.2.5 Inactivate micro-organisms and enzymes

Food preservation by elevated temperature for short period of time is still the most common form of food preservation process. In most cases the process variables and controls have been derived by empirical investigation of the effect of temperature and time of exposure

on microbial survival kinetics with little regard given to food quality in relation to thermal effects on food composition and structure. The loss of quality is brought about by deformation of plant and animal structures, modification of macromolecules and the production of new substances from heat-catalyzed reactions. Ultrasound offers some exciting opportunities to reduce these effects. The high temperatures to which microbial cells are exposed will not affect one specific target, as the thermal energy in the cell is an integral part of an entire complex system. The heat energy affects a wide variety of cellular constituents, including structures, molecules and the reactions in the cell. These targets provide opportunities to harness the cell damaging ability of ultrasound for combined preservation systems.

The application of ultrasonic waves generating cavitation in suspensions, which contain micro-organisms and enzymes, often has a lethal result and deactivating action (Suslick, 1988). When high power ultrasound propagates into a liquid the micro-bubbles, which are commonly present in it or that may form from the presence of suspended particles, will oscillate according to the pressure wave. High acoustic pressure will determine their growth and violent collapse, which is accompanied by a sudden increase of the temperature and the pressure in the surrounding area.

4.1.2.6 Effect on rice grains

If the particles subjected to sonication are rice grains in water then some destruction of surface “shell” and grain fragmentation would be anticipated. Both of these effects would result in a faster release of starch during cooking leading to a shorter period to form a gel (Mason et al., 1996).

4.1.2.7 Accelerate extraction processes

Ultrasound assists extraction processes. The classical techniques for solvent extraction of materials from vegetable sources are based upon the correct choice of solvent coupled with the use of heat and/or agitation. The extraction of organic compounds contained within the body of plants and seeds by a solvent is significantly improved by the use of power

ultrasound. The mechanical effects of ultrasound provide a greater penetration of solvent into cellular materials and improve mass transfer. There is an additional benefit for the use of power ultrasound in extractive processes, which results from the disruption of biological cell walls to facilitate the release of contents. This has been shown in a study from sugar beet (Mason et al., 1996). Chymosin and some other enzymes soluble in sodium chloride solution are extracted from calf abomasa. The current commercial extraction process has been improved to maximize recovery of chymosin and minimize presence of other protease and lipase (Barbono, 1986). Ultrasound may be the simplest and most versatile method of breaking cells and preparing extracts. The release of enzymes and proteins from cells and subcellular particles is a unique and effective application of ultrasound, which causes destruction of cellular structure by a cavitation effect (Kim and Zayas, 1991).

Protein extraction from defatted soya beans was studied by Povey and Mason (1998). A continuous process was developed where sonification of the slurry by a 550 W probe operating at 20 kHz frequencies resulted in an efficient extraction which exceeded any previously available technology. This was scaled up to pilot plant level for the extraction of soya bean protein (Povey and Mason, 1998).

4.1.2.8 Meat products

Closely linked with extraction is the methodology employed for the production of meats. Generally this involves tumbling the meat particles with an aqueous liquor containing salt. Ultrasound assists the process by disrupting the meat myofibrils, which releases sticky exudates, and this binds the meat together and leads to an increase in the strength of the reformed product. The binding strength, water holding capacity, product colour and yields were examined after treatment either with salt tumbling, sonication or both. Samples, which received both salt treatment and sonication, were superior in all relevant quality. A study of the effect of sonication on cured rolled ham showed similar result. A traditional method of tenderisation of meat is by mechanical pounding, which makes poor quality meat more palatable. Sonication of steak has also been found to be useful in the tenderisation process.

4.1.2.9 Emulsification

One of the earliest uses of power ultrasound in processing was in emulsification (Povey and Mason, 1998). If a bubble collapses near the phase boundary of two immiscible liquids the resultant shock wave can provide a very efficient mixing of the layers. Stable emulsions generated with ultrasound have been used in the textile, cosmetic, pharmaceutical and food industries. Such emulsions are often more stable than those produced conventionally and often require little, if any, surfactant. Emulsions with smaller droplet sizes within a narrow size distribution are obtained, when compared to other methods. The degree of emulsification in such materials can also be estimated by the measurement of ultrasound velocity in conjunction with attenuation. It is possible to determine factors such as the degree of "creaming" (or "settling") of a sample, i.e. the movement of solid particles/fat droplets to the surface (or to the base). Such information gives details, for example, of the long-term stability of fruit juices and the stability of emulsions such as mayonnaise. The combination of velocity and attenuation measurements shows promise as a method for the analysis of edible fats and oils and for the determination of the extent of crystallization and melting in dispersed emulsion droplets.

4.2 Potential of ultrasound in lemon juice industry

4.2.1 General consideration

The most frequent reason for the deterioration of lemon products is the development of microbial activity and this often results in moulding, fermentation and acidity. Spoilage of lemon juice is mostly caused by aciduric micro-organisms such as *Leuconostoc* species (Uelgen and Oezilgen, 1993). In addition there may be enzymatic transformations due to enzymes of the juice itself or those produced by micro-organisms. For the lemon juice product, thermal treatments are generally employed and should be sufficient to inactivate both microbes and enzymes, in particular pectinesterase (PE; pectinmethylesterase, pectase, pectinmethoxylase, pectin pectilhydrolase, EC 3.1.1.11) and polygalacturonase (PG; polygalacturonidase, pectinase, pectolase, and pectin-polygalacturonidase, EC 1.2.1.15)

activities. Recent research has shown that PG is not the primary determinant of citrus fruit softening (Gross, 1991; Ketsa and Daengkanit, 1999). Therefore, PG itself may not be sufficient to induce cloud loss of the lemon juice. Besides, PE is the most heat-resistant cloud-destabilizing enzyme present in lemon juices. Inactivation of PE is generally used as an indicator for the adequacy of pasteurization because it is known to be more heat resistant than the common micro-organisms. Ascorbic acid is the important nutrient in lemon juice, but it undergoes degradation during pasteurization and storage.

Inactivation rates of micro-organisms and enzymes and degradation rates of nutrients strongly depend on the pH and processing temperatures. A food processing system capable of adjusting the pH of juice by blending or substituting one citrus juice with others allows the optimum pasteurization pH and temperature for assuring maximum ascorbic acid retention while inactivating sufficiently micro-organisms and enzymes. There are numerous examples in the literature of optimization of the sterilization process for maximum nutrient retention by varying both pH and temperature (Uelgen and Oezilgen, 1993).

Although synthetic clouding agents have been used for years, recently several have been barred or their use restricted below a useful level. Therefore, from a legal perspective, a natural clouding would be preferred to synthetic agents. When citrus fruit are juiced, about 50% of their weight is left as waste peel, membranes, juice vesicles and seeds. One of the most promising products that can be made from this waste is a natural beverage clouding agent. The production of this new agent is also desirable because it helps to eliminate one source of pollution during citrus manufacturing (El-Shamei, Z. and El-Zoghbi, M.; 1994).

One problem in lemon juice pasteurization is that the high temperature results in flavour and aroma changes as well as losses in vitamins and volatile compounds. Inaddition, heat promotes browning reactions in the citrus juice. The magnitude of these changes increases with the increasing of time and temperature. The development of new technological procedures that would be more efficient for pasteurization would be a great advantage for lemon juice industry.

4.2.2 Conventional treatment for lemon pectinesterase inactivation

In order to prevent cloud loss, citrus juice is pasteurized at high temperature (90°C for 1 min) to inactivate PE (Eagermann and Rouse, 1976; Versteeg, 1979). Versteeg et al. (1979) demonstrated that citrus contains two isozymes of PE, which are heat labile at 70°C and a third isozyme, which is stable at temperatures up to 80°C. They also showed that cloud loss was due to improper inactivation of this heat stable isozyme. Since the inactivation temperature in this study was at 90°C, only the heat stable PE isozyme was of interest. Eagermann and Rouse (1976) examined the PE inactivation in juices extracted from different citrus varieties and found that the *z*-values ranged from 4.4-6.7°C depending on variety. PE inactivation was faster at low pH values than at higher values (Atkins and Rouse, 1953; Kew et al., 1957; Draetta et al., 1979). Nath and Ranganna (1977) showed that the processing time for PE inactivation increased as the pH increased. They demonstrated that juice stabilization could be achieved by processing 2D value at pH 3.6, while a 2.5D would be required to stabilize juice at pH 4.0. Moreover, the inactivation of PE was faster in high Brix than in single strength juices at 68.3°C (Atkins et al., 1956). However, at higher temperature (73.8°C and greater) erratic results occurred, indicating that PE inactivation was not faster in the higher concentration (Marshall, Marcy and Braddock, 1985). Braddock suggested that a decrease in the PE inactivation rate occurred indicated that the inactivation of PE may be protected at higher Brix. Increasing the enzyme concentration 4-fold showed that an S-shaped or sigmoidal curve resulted.

4.2.3 Potential of ultrasonic application in the lemon juice industry

Ultrasound processing in combination with lower heat process temperatures has many advantages. It can lead to better quality products, with improvements in taste, texture and appearance. It also could result in reduced energy requirements and therefore reduced cost. The application of ultrasound with heat will require the design of new types of processing equipment. The design of heat exchangers and heating system, which incorporate powerful ultrasonic transducers, will not be easy. The fact that heat can be applied after ultrasound

treatment with useful effects is encouraging if one considers the engineering aspects. The compatibility of ultrasound processing with existing material handling and packaging systems is encouraging. Plant modification will be needed rather than complete equipment replacement: this has significance in terms of commercial realization and the demands on capital investment needed to put the technology to work. The likely applications are difficult to predict at this stage but thermosonication and manothermosonication is particularly suited to pumpable liquids and liquid containing solids, which could be processed in a continuous flow arrangement with clean or aseptic filling. The prospect of applying ultrasound to foods already packed into containers is conceptually possible but the practical constraints need to be considered in details, particular in terms of ultrasound penetration. Although the possibility of deactivating enzymes or destroying micro-organisms by ultrasound waves, alone or in combination with other physical treatments, has been widely used for laboratory applications in microbiology, immunology and enzymology, it is not true for industrial applications. The reasons of the non-development on an industrial scale of this technique are numerous and in part the non-development is due to the lack of information needed for design and scale-up procedure (Mason, Lorimer and Bates, 1992).

Table 4.1: Literature summary of citrus pectinesterase inactivation and ultrasonic application on enzyme inactivation

Researcher	Methods	Enzymes	Year	Finding			
				D-value (min)	z-value (°C)	E_a (kJ/mol)	Note
Eagerman and Rouse	T	Citrus PE (hamlin, pineapple, orange)	1976		12.2°F		The necessary pasteurization temperature, F- and z-value for PE inactivation were developed
Marshall et al.	T, pH, solid level	Fresh orange PE	1985	18 (pH 4) 7 (pH 7)			PE heat inactivation at various Brix did not obey first-order reaction kinetics. At high Brix levels, a protective effect seemed to occur because the rate at enzyme inactivation declined.
Wicker & Temelli	T	Orange PE	1988	0.225 (heat labile) 32 (heat stable)	10.8 (heat labile) 6.5 (heat stable)		Heat inactivation was non-linear. The residual activity rapidly decrease to 4% upon heating for 19 sec at 80°C.
Balaban et al.	Super critical CO_2	Orange PE	1991	D_{60} = 56.6	8.8	166.6	High pressure CO_2 can inactivate PE depended on P, T and time
De Sio et al.	T	Tomato PE	1994	0.17-0.19	11.2		The inactivation was found to be exponential.
Lopez et al.	MTS	Horseradish POD, mushroom PPO, soybean Lipoxygenase	1994		26 (POD) 6.8 (Lipoxygenase)		The enzyme destruction efficiency of combined process greatly increases with US amplitude.
Andrew et al.	pH variation	Tomato PE	1995				Non-linear regression and the behavior of all three forms of enzyme generally showed good fit to the Michaelis-Menten equation.

Researcher	Methods	Enzymes	Year	Finding			
				***D*-value (min)**	***z*-value (°C)**	**E_a (kJ/mol)**	**Note**
Basak & Ramaswamy	HP	Fresh orange PE	1996	145 (pH 3.2, $4x10^5$ kPa) 260 (pH 3.7, $4x10^5$ kPa)			Inactivation of PE orange was depended on pressure level, holding time, pH and soluble solid
Vercet et al.	T and MTS	Protease and Lipase from *Pseudomanas fluorescen*	1997	13 (T, Protease) 7.5 (T, Lipase) 7.5 (Lipase, 450 kPa, 30°C) 4.5 (Protease, 450 kPa, 30°C)	31.2 (T, Protease) 47.6 (MTS, 450 kPa, 145 µm, Protease)	950 (T, Protease) 62.7 (MTS, Protease)	The synergistic effect occurs due to the combination of heat and US under pressure. The decrease of MTS efficiency in lipase at high temp. due to loss of cavitation intensity. A single mechanism is responsible for heat and MTS protease inactivation. Two mechanisms operate lipase in MTS inactivation.
Thakur & Nelson	US	Lipoxygenase	1997				Inactivation of the enzyme was influenced by the time of exposure, pH, and amplitude of ultrasound.
Cano et al.	T, HP	POD, PPO, PE	1997				Increased soluble solids protect PE against pressure.
Arbaisah et al.	T	Citrus PE	1997	D_{65} (PEI) = 5.8 D_{65} (PEII) = 3.3	8.5 (PEI) 8.6 (PEII)		Cloud destabilization by PEI occurred fastest in the natural juice at 30°C.
Hernandez & Cano	HP	POD, PPO, PE (tomato)	1998				Increased soluble solids protect PE against pressure as well as heat inactivation.
Goodner	HP	Orange PE	1999				Pressure $8x10^5$-$9x10^5$ kPa were much more effective in preserving cloud at shorter processing time

Researcher	Methods	Enzymes	Year	Finding			
				D-value (min)	**z-value (°C)**	**E_a (kJ/mol)**	**Note**
Gennaro et al.	MTS	Horseradish POD	1999	$D_{80} = 10$			The POD deactivation due to ultrasound treatment follows first order kinetics. The most efficient conditions for combined heat & ultrasound treatment in batch mode seem to correspond to a frequency of 20 kHz and a power density close to 0.6.
Vercet et al.	MTS	Orange PE	1999		35.7	56.9	The effect of heat and US waves in enzyme combine synergistically.

Part III

Process

Chapter 5
Materials and methods

5.1 Juice preparation

5.1.1 Preparation of lemon juice

For the experiment of discontinuous process, the 9 kg whole Spanish lemons were washed at 4°C and cut into little pieces by household mixer (Braun, Germany type 3210) at the middle speed for 30 minutes. The slurry was blended with 1 M Tris (8 litre) containing 0.1 M in NaCl (pH 8.15) at 4°C for 90 min. The solution was filtered through muslin. The filtrate was added with ammonium sulphate (25% saturation) and centrifuged at 11600 x g at 0°C for 20 min to remove some pectin. The supernatant was added with 80% saturation of ammonium sulphate and left overnight. The solution was centrifuged at 11600 x g at 0°C for 20 min. The supernatant was added with 10% water weight and centrifuged at 11600 x g for 20 min. The pellet was redissolved with 900 ml Tris HCl (pH 7) containing 0.1 M in NaCl and left overnight. The solution was then centrifuged at 12000 x g, 20 min. The supernatant was precipitated with 80% saturation of ammonium sulphate. The solution was centrifuged again at 12000 x g for 20 min. The pellet was diluted with Tris HCl (pH 7.0) 0.1 Min NaCl (~300 ml). The extract had approximately 64650 units of PE activity.

For the experiment of continuous process the lemons from Spain were purchased from a local store (Berlin). 20 kg freshly chopped lemons were blended at 4°C with 1 M Tris (25 litre) containing 1 M NaCl. The slurry (pH > 8) was allowed to stand for 90 min, before pressing through muslin. The filtrate was very viscous owing to the presence of pectin. The filtrate was then added with ammonium sulphate (to 25% saturation) and centrifuged (11500 x g, 15 min) to remove some of pectin. The solution was left overnight. Protein and PE precipitation was made by addition of the ammonium sulphate (to 80% saturation). After centrifugation (11500 x g, 15 min), the gelatinous pellet was redissolved in 2 litre of Tris HCl (pH 7.0) with 0.1 M in NaCl and left overnight to allow precipitation of pectates produced by PE action. After centrifugation (11500 x g, 15 min), the supernatant was stored at -20°C until required. The yield of PE was 104500 Units. The freeze extracted samples containing PE enzyme were redissolved in distilled water, pH 7.0 and heated at 70°C for 5 min to obtain only heat-stable portion of enzyme. Then the activity of enzyme solution was measured at various temperatures and duration.

For the experiment in the pilot plant the 250 kg of Spanish lemons were squeezed by a household squeezer (Braun, Germany) and filtered through muslin in order to have clear juice. Juice was maintained at temperature approximately 4°C by subjecting the ice bags to the container. The juice has initial pH 2.45 and light green-yellow colour before applying to the process system.

5.1.2 Preparation of strawberry juice

Frozen strawberries (60 kg) were obtained from the strawberry juice company (TYC, Poland). The strawberries for the processing were selected to be mature and disease free. Strawberry puree, obtained by homogenization using a blender (Stephan MicRoCut; MC H 20 k; STEPHAN und Söhne Co. Hameln, Germany), was depectinized by the enzyme solution (Pectinex Ultra SP-L; Novo Nordisk Ferment Ltd., Switzerland) (Dosis 0.5%; at 50°C for 30 min) in order to achieve maximum juice yield. The juice was filtered through muslin to obtain clear juice before applying to the MTS treatment system. The treatment process was summarized by the flow chart in Figure 5.1.

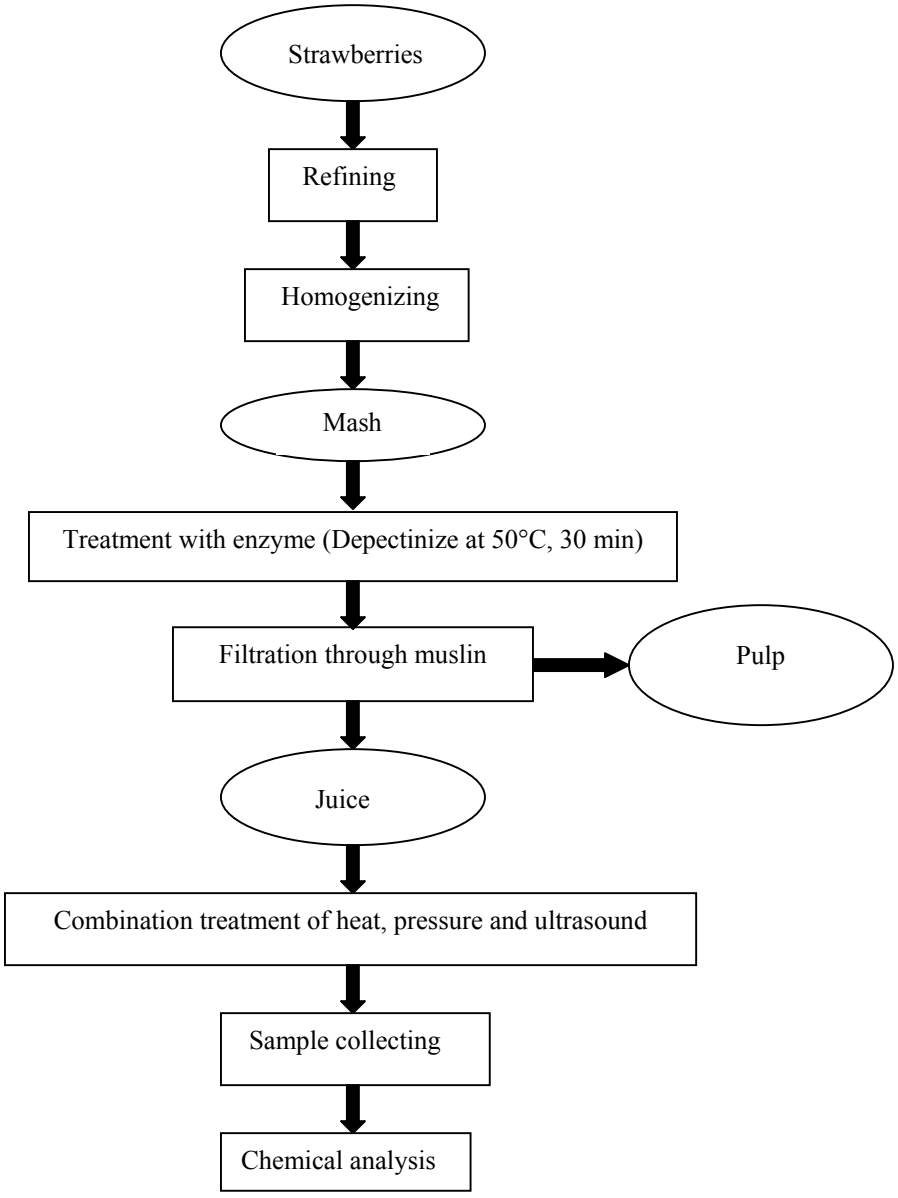


Figure 5.1: Flow chart of the strawberry process

5.1.3 Preparation of tomato juice

14 kg fresh tomatoes (ALP Co., Turkey) were purchased from a local shop. Fresh tomatoes were mixed with cold 1 M NaCl solution (4°C) in the proportion 1:1 in the household blender (Braun, type 3210-645, Germany) for 30 sec with maximum speed. The slurry was stored in a large container with ice bags (~ 4°C). The solution was centrifuged at 3200 x g for 5 min (Minifuge RF; Heraeus Sepatech, Germany). The supernatant was dialyzed through the dialyze tube (pore diameter 0.0025-0.005 μm, cut off 10000-20000, Roth,

Karlsruhe) and left in the distilled water (4°C; solution : water = 1:15) for 3 hours. The conductivity of the solution after the dialyzation was 5.1 mS/cm. The dialyzed solution was kept in polyethylene bags and stored at -20°C.

5.2 Equipment and experiment set-up

5.2.1 Discontinuous unit

A submerged ultrasonic horn for 100 µl - 50 ml, with a tip diameter of 3 mm and fixed frequency 20 kHz, was used. A generator (Bandelin Electronic, Berlin) converts 50 Hz electrical energy into 20 kHz. A transducer containing the piezo-electronic element enabled the conversion of 20 kHz electrical energy to vibrating mechanical energy of the same frequency.

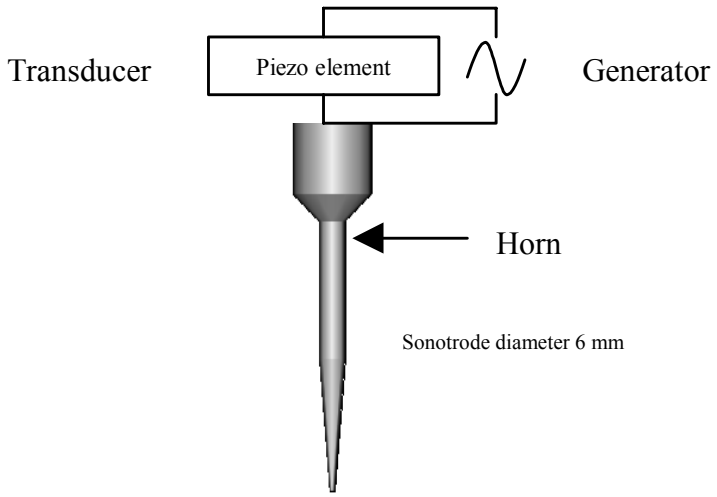


Figure 5.2: Ultrasonic horn 20 kHz

Experiment performed in water bath adjusting temperature to 40°C-90°C. Two thermometers were placed either in solution and water bath to observe the temperature profile. Time was started measuring as soon as the temperature of the solution reached the water bath temperature.

Table 5.1 Actual power and amplitude of the ultrasonication in discontinuous unit

Ultrasonic power (%)	Actual power (W)	Amplitude (μm)
20	6.5	36
40	10.5	-
60	15.5	108
80	22.0	-
100	25.0	162

5.2.2 Continuous unit

The ultrasonic treatment was performed in the continuous ultrasonic system (Dr. Hielscher, Berlin, Germany). Table 5.2 presents the technical data of the ultrasonic equipment.

Table 5.2 Ultrasonic equipment in the continuous process

Sonotrode	Sonotrodetype: S14 with the non-vibration flange. Sonotrode is made from titanium, diameter 14 mm, suitable to operate under pressure $\leq 10^3$ kPa
Ultrasound processor	200 Watt, frequency 24 kHz, automatic frequency scanning-system, power controlled from 20-100 %, pulse controller 0-100% (It was not used in the experiment), amplitude 100% = 35 μm, neutral power 100% = 25 Watt
Flow system	High-grade steel, autoclavable, attached by flanges that resisted to the pressure $\leq 10^3$ kPa, the total volume approximately 10 ml

The piston pump (Knauer, HPLC pump, type 6400, Berlin) drew the sample through the system. The flow rate was adjusted to keep the flow continuous. For the

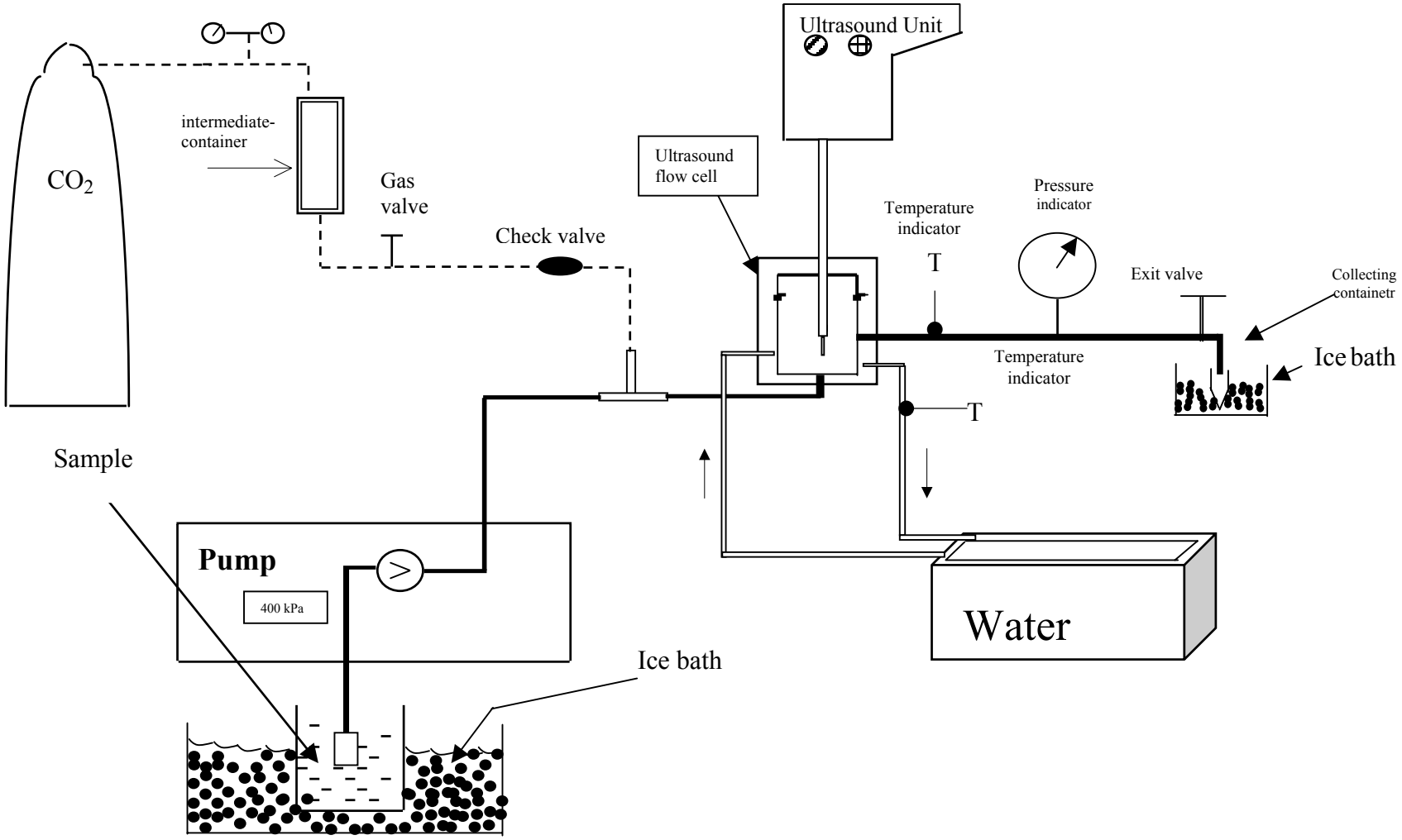
operation with pressure 400 kPa, the outlet valve was controlled to maintain the constant pressure. Figure 5.3 presents the equipment set up. The combination treatment of ultrasound and CO_2 was also carried out in the same equipments. The pressure was considered to be the pressure of CO_2. The temperature of the sample during the experiment was constantly controlled by the water bath. The temperature profiles were also recorded during the experiment. Table 5.4 and Table 5.5 are the experiment conditions, which show the treatment temperature, pressure, time for the treatment with and without ultrasound. The ultrasound energy is also depended on treatment temperature and pressure (Figure 5.5 and Figure 5.6). Increased pressure dramatically increases the ultrasound power (Figure 5.5). Contrarily, the increased temperature slightly decreases the ultrasound power (Figure 5.6).

Sonotrode	
Power:	200 W
Ultrasonic power:	20% - 100%
Frequency:	24 kHz
Dimension:	190 x 200 x 90 mm^3 (Length x Width x Height)
Weight:	2.3 kg
Generator	
Electricity:	230 V, 8 A, 50-60 Hz
	115 V, 16 A, 50-60 Hz

Table 5.3: Actual power and amplitude of the ultrasonication in continuous unit

Ultrasonic power (%)	Actual power (W)	Amplitude (μm)
20	33.5	7
40	69.5	14
60	104.5	21
80	138.0	28
100	175.0	35

Figure 5.3: The continuous MTS equipment and the alternative process of the combination treatment with CO_2



5.2.2.1 Thermosonication

Heat treatments were performed between 40°C - 80°C in the treatment chamber surrounded by the cooling jacket. The flow rate was controlled by the HPLC. The holding time was converted from the HPLC flow rate chart. The ultrasound power was adjusted to 100% to perform the maximum reaction.

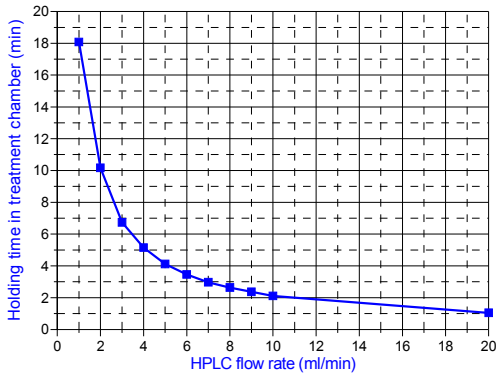


Figure 5.4: The calibration curve of the holding time vs. the HPLC flow-rate

Table 5.4: Temperature and actual power during thermal treatment and thermosonication (without pressure)

		Thermal treatment			Thermosonication		
Temperature (°C)	Treatment time (min)	Temperature in water bath (°C)	Temperature of the sample after treatment (°C)	Power (W)	Temperature in water bath (°C)	Temperature of the sample after treatment (°C)	Power recorded during the treatment (W)
40	1.0	41.0	39.0	—	36.0	39.3	63.0
	2.0	41.0	41.0	—	34.0	39.2	63.0
	4.0	41.0	41.0	—	33.0	39.6	65.0
	5.0	41.0	41.0	—	33.0	39.4	63.0
50	1.0	50.0	50.0	—	46.0	50.5	63.0
	2.0	51.0	51.0	—	—	—	—
	4.0	52.5	51.0	—	45.0	49.7	61.0
	5.0	56.0	49.0	—	50.0	49,9	61.0
60	0.5	62.0	61.0	—	57.0	60.2	57.0
	1.0	62.0	60.0	—	56.0	60.0	56.0
	2.0	62.0	60.0	—	58.0	60.6	59.0
	4.0	63.0	60.0	—	61.0	60.0	59.0
	5.0	69.0	59.5	—	63.0	60.0	61.0
70	0.5	73.5	70.5	—	68.0	70.5	54.0
	1.0	73.0	70.0	—	—	—	—
	2.0	74.0	71.0	—	69.0	70.6	54.0
	4.0	76.0	71.0	—	71.0	70.0	56.0
	5.0	79.0	70.5	—	78.0	70.0	56.0
80	0.5	83.0	78.5	—	—	—	—
	1.0	84.0	81.0	—	81.0	80.0	36.0
	2.0	84.0	81.0	—	80.0	79.5	43.0
	4.0	86.0	78.0	—	83.0	80.0	50.5
	5.0	91.0	74.0	—	88.0	79.1	49.0
90	0.5	94.0	90.0	—	—	—	—
	1.0	96.0	90.0	—	92.0	90.0	42.0
	2.0	99.0	91.0	—	96.0	90.0	45.0
	5.0	99.0	80.5	—	101.0	89.0	45.0

5.2.2.2 Manothermosonication

Manothermosonication was performed in the treatment chamber surrounded by the cooling jacket. The operating temperatures were set from 40°C to 80°C. Inside the vessel (10 ml), the treatment chamber was fitted with the sonotrode model UP200S from Dr. Hielscher GmbH, Germany. The ultrasonic horn irradiates at a fixed frequency of 20 kHz. Pump HPLC allowed the enzyme solution to the treatment chamber at the adjusted flow rate. The pressure (100 - 300 kPa) was manually controlled by the outlet valve.

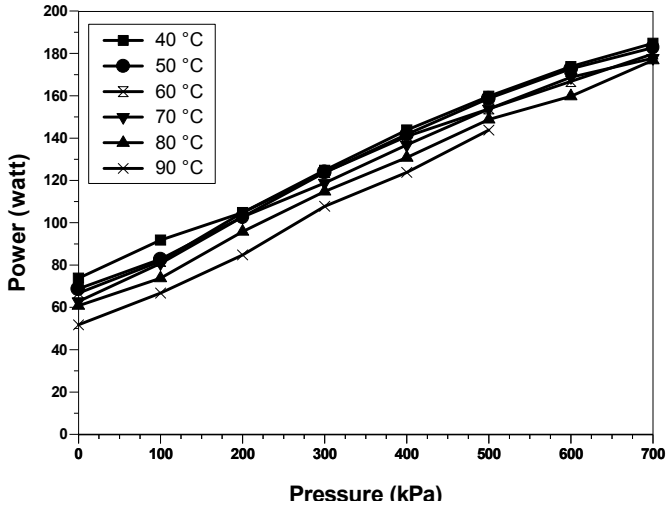


Figure 5.5: Ultrasonication power during pressure treatment

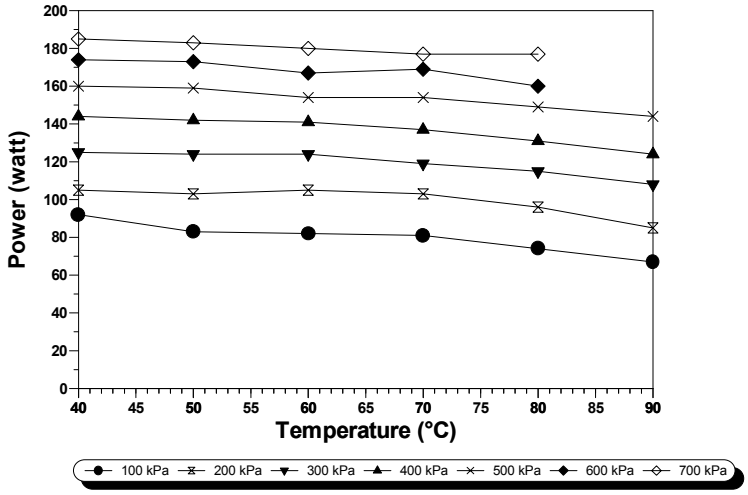


Figure 5.6: Ultrasonication power during thermal treatment

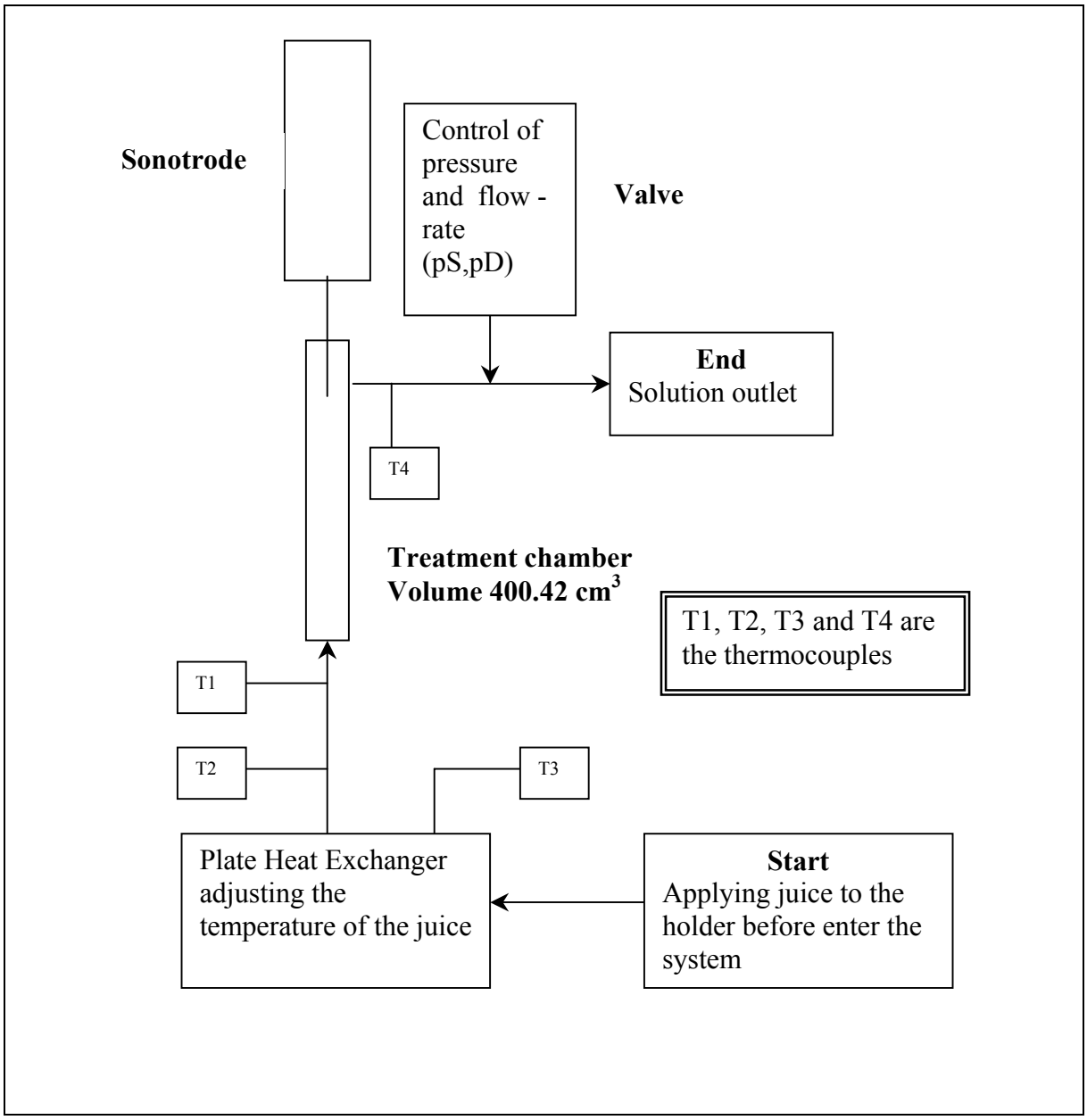
Table 5.5: Temperature and power during the combination treatment of temperature and pressure and manothermosonication

		Thermal treatment under 400 kPa			Thermosonication under 400 kPa		
Temperature (°C)	Treatment time (min)	Temperature in water bath (°C)	Temperature of the sample after treatment (°C)	Power (W)	Temperature in water bath (°C)	Temperature of the sample after treatment (°C)	Power recorded during the treatment (W)
40	1.0	40.0	40.0	—	19.0	39.0	139.0
	2.0	40.0	40.0	—	26.5	41.0	136.5
	4.0	40.0	40.0	—	29.5	45.0	135.0
	5.0	42.0	40.0	—	32.0	46.0	135.0
50	1.0	52.0	50.0	—	33.5	50.0	135.0
	2.0	50.5	50.0	—	33.0	49.0	135.0
	4.0	50.5	50.0	—	35.0	50.0	135.0
	5.0	54.0	50.0	—	36.0	49.5	135.0
60	1.0	63.0	60.0	—	46.0	60.0	143.0
	2.0	61.5	60.0	—	46.0	60.0	133.0
	4.0	62.5	60.0	—	46.0	60.5	133.0
	5.0	64.5	60.5	—	51.0	60.5	133.0
70	1.0	73.0	70.0	—	57.0	69.0	133.0
	2.0	73.0	70.0	—	57.0	69.0	131.0
	4.0	74.0	71.0	—	58.0	70.0	132.0
	5.0	76.0	71.0	—	60.0	69.0	130.0
80	0.5	85.0	80.0	—	70.0	79.5	125.0
	1.0	83.0	80.0	—	70.0	79.0	126.0
	2.5	89.0	80.5	—	74.0	80.0	126.0
	4.0	100.0	79.5	—	77.0	80.5	125.0
90	0.5	97.0	90.0	—	80.0	89.5	125.0
	1.0	97.0	90.0	—	82.0	90.0	125.0
	2.5	102.0	83.0	—	86.5	91.0	125.0

5.2.3 Pilot plant unit

The experiments were performed in a continuous system combining of temperature, pressure and ultrasound (Zenker et al., 1999).

Figure 5.7: The continuous system of MTS (400.42 cm^3 treatment volume)



The sonotrode (UIP 1000 Ultrasound-Industrial processor 1000 W, Dr. Hielscher Co., Ltd., Berlin) was manipulated in the experiment.

The technical data are as follows:

Sonotrode	
Power:	1000 W
Usual power:	20% - 100%
Frequency:	20kHz
Maximum amplitude:	35 – 45 μm
Dimension:	112 x 71 x 440 mm^3 (Length x Width x Height)
Flange-diameter:	100 mm
Generator	
Electricity:	230 V, 8 A, 50-60 Hz
	115 V, 16 A, 50-60 Hz
19"-System:	363 x 365 x 153 mm^3 (Length x Width x Height) of the case.

Zenker et al. (1999) also stated that the ultrasonic power was slightly decreased from 700 W to 650 W when the temperature of the medium increased from 30°C to 80°C at 300 kPa.

Juice was held in the holding tank before entering the system by the controlling valve. The closed valve allows the system initially operated with the distilled water in order to adjust the required temperature, pressure and flow rate. After the system reached the equilibrium, the juice was necessary to be subjected to the system by the immediate opening of the valve; otherwise a huge pressure drop may occur.

The juice was heated up to the required temperature by passing through the heat exchanger. The temperature was shown at the outlet of the heat exchanger (T2). The flow rate was adjusted through out the experiment in order to keep the pressure constant, which functioned by opening and closing the ventilation valve at the outlet. In this experiment, the pressure was stable at 300 kPa.

Four thermometers were installed at the inlet (T3) and the outlet (T2) of the heat exchanger, inlet (T1) of the treatment chamber and at the outlet (T4). The temperatures were measured through out the process. The outlet temperature (T4) was taken into account as the control temperature.

Table 5.6: Mass flow and the holding time in the equipment

Mass flow (kg/h)	holding time in the treatment chamber (sec)	Holding time in the outlet path (sec)	Holding time before entering the treatment chamber (sec)
60	27.2	25.036	22.2
50	32.6	30.1	26.6
40	40.3	37.63	33.3
30	54.4	50.17	44.4
20	81.7	75.25	66.6
10	160.4		

After the heating section, the temperature of the juice slightly decreased due to the energy loss before entering the adjacent area of the sonotrode. The advantage of the decreasing of the temperature is to overcome the desired value of temperature after sonification. The temperature was then again significantly increased during the sonification. The process pressure was simultaneously controlled by the ventilation valve (at the outlet) and the hydraulic pump. Sizes of the pumps also affected the pressure control. Large pump could give more stable value of high pressure rather than small pump. In contrary, the small pump controlled low pressure more successfully than large pump.

The experiments were operated at different temperatures and different powers of sonification 0%, 20%, 50% and 100%. These different powers of sonification gave different value of circuit power (Watt) (see Appendix A.10 for the lemon juice and Appendix A.13 for the strawberry juice). The actual values of power were recorded during the treatment. The pressure was constantly maintained at 300 kPa and most of the experiments were run at approximately 10 L/h (Appendix A.10 for the lemon juice and Appendix A.13 for the strawberry juice).

5.3 Analysis

5.3.1 Pectinesterase assay by acid-base titration

The results from the discontinuous system and the pilot plant were analyzed by method of titration. This method is easy to adjust the pH value of the samples. Many researchers have also analyzed the PE activities by this method (Vercet et al., 1999; Macdonald et al., 1993; Sio et al., 1995; Hernandez et al., 1998; Laratta et al., 1995; Lopez et al., 1998).

The pH of titration was constantly maintained at 7.0, which enhanced the activation of pectinesterase to the highest level. A unit of PE activity is defined as one μmol of carboxyl group produced per minute. Methods of titration are as follows.

50 ml of 1% Pectin weight concentration (Pomosin Co., GENU Pectin, Type B rapid Set-Z) (adjusted pH by 0.1 M NaOH) was added in double glass layer circulated with water 30°C and stirred by small agitator. The pH was shown on the monitor of the titrator by the attached pH indicator.

1 ml of PE was applied. The pH of pectin will decrease due to the result of PE activity. The pH of 7.0 was manually adjusted by applying 0.01 M NaOH and time was started to record. The amount of used NaOH per minute was recorded for 10-15 min. The average amount of NaOH per min is the activity of the enzyme according to the following equation:

$$PE\ Unit = \frac{(ml\ of\ used\ NaOH)(mol/l\ of\ NaOH)(10^3)}{(ml\ of\ applied\ PE)(time\ (min))} \qquad (5.1)$$

5.3.2 Pectinesterase assay by method of spectrophotometry

The existing methods of titration require large volumes of reactants and are time consuming. A spectrophotometry for pectinesterase is convenient, sensitive and specific (Hagerman A.E., Austin P.J.; 1986).

The spectrophotometric assay for pectinesterase is based on the colour change of a pH indicator during the PE-catalyzed reaction. The method determines PE activity by the measurement of H+ ions released from PE mediated demethylation of pectin using a pH indicator. The method further develops using the more sensitive pH indicator phenol red (Randall et. al, 1992), instead of bromothymol blue (Hagerman A.E., Austin P.J.; 1986). Bromothymol blue was found to have a 3x lower sensitivity relative to phenol red.

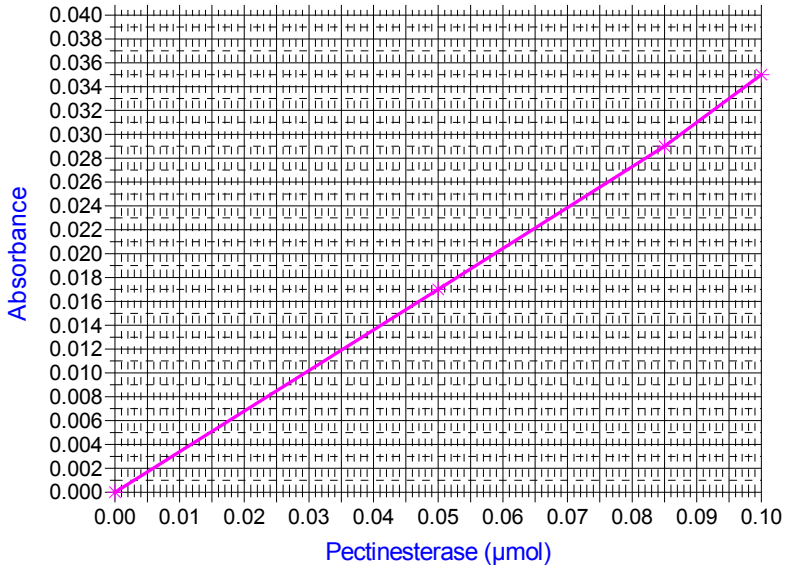


Figure 5.8: The calibration curve of sprectrophotometry with phenol red

PE enzyme was extracted from lemon pulp and peel and a series of enzyme dilution made. These dilutions were used to calibrate the plate reader by calculating PE activity from the titration and comparing them to the rate colour change of the pH indicator on the spectrophotometry.

The assay must be started at the same pH 7.5 and the buffer interfere with the measurement of acid production, therefore the reagent must be prepared as weakly buffered solution. The methods are as followed:

Pectin, indicator dye, water were adjusted to pH 7.5 with 1M NaOH. Citrus pectin (Pomosin Co., GENU Pectin, Type B rapid Set-Z) (0.5% w/v) was prepared in distilled water and adjusted to pH 7.5. 0.01% w/v of phenol red was prepared in 0.003 M potassium

phosphate buffer (pH 7.5). Pectinesterase was previously prepared in distilled water pH 7.5. The absorbance was monitored at 555 nm in recording spectrophotometer (Hitachi Co., Japan). Temperature was maintained 25°C with circulating bath. The glass cuvette of 3 ml was filled by 0.15 ml phenol red and 0.85 ml distilled water and 2 ml pectin solution (0.5% w/v). The initial absorbance at 555 nm of the mixture was determined and used as the reference. The next cuvette (0.15 ml of phenol red, 0.8 ml of distilled water, 2 ml of pectin and 50 µl of treated PE) was measured and compared to the reference by the rate of decrease in absorbance per minute. The spectrophotometry was calibrated with galacturonic acid (Sigma) and natural lemon pectinesterase. In cuvette 2 ml of pectin was mixed with 0.15 of phenol red and 0.1 to 0.85 ml of water and acid (or pectinesterase) to bring the volume of 3 ml. The rate of absorption per minute was used to determine the actual activity (µmol/min) of pectinesterase with respect to the calibration curve.

The experiment of tomato juice, pectinesterase activity was obtained by the photometric method of Hagerman and Austin (1986) (620 nm). 2.8 ml of 0.5% pectin (pH 7.5; Pomosin Co., GENU Pectin, Type B rapid Set-Z) was applied into 100 mM NaCl and 0.01% (W/V) NaN_3. The 0.025% (W/V) bromthymol blue solution (BTB) in 3 mM calcium phosphate (pH 7.5) was set as the colour reagent. The measurement was made by the composition of 2 ml pectin solution, 0.65 ml distilled water (pH 7.5), 0.3 ml BTB and 0.05 ml sample.

5.3.3 Analysis of other properties

5.3.3.1 Polyphenoloxidase (PPO) assay

The method of PPO analysis follows the photometry method of Hernandez and Cano (1998). 2 ml of 0.15 M catechol solution in 0.05 M calciumphosphate buffer (pH 6.5) was applied in the glass cuvette. 1 ml of sample was also applied and mixed with the previous solution. The enzyme activity was determined by the photometer (Hitachi, Japan) at 420 nm and 25°C.

5.3.3.2 Peroxidase (POD) assay

The POD activity was obtained through the photometry method at 420 nm and 25°C (Method by Sigma Co.). 0.32 ml of phosphate buffer (pH 6), 0.16 ml hydrogen peroxide (1.67 ml of 30% of the solution diluted to 100 ml), 0.32 ml of 5% pyrogallol solution (Merck, Germany) and 1.1 ml of distilled water were applied into a glass cuvette. 1 ml of the sample was applied. The calibration value was obtained by applying 1 ml of the distilled water instead of the sample.

5.3.3.3 Polygalacturonase (PG) assay

The polygalacturonase activity was determined by the method of Honda et al. (1982). 0.25 ml of the sample was applied into the reagent glass as well as 0.15 ml of 0.4% polygalacturonic acid (Sigma Co.) (washed in 80% Ethanol and dried at 40°C). The reagent glass was incubated at 40°C in water bath for 2 hours. After the incubation, 1 ml of 1% 2-cyanoacetamid (Aldrich Co., Germany) and 2 ml of 0.1 M borat buffer (pH 9) were applied into the reagent glass. The reagent glass was heated at 100°C in hot water for 10 min. Then the reagent glass was cooled at room temperature. The absorbance was photometrically measured at 276 nm comparing to the calibrated solution. This calibrated solution was prepared following the same method as the sample preparation, but without incubating at 40°C.

5.3.3.4 Total dry solids dissolved in the juice (Brix value)

The measurement of dry substances was carried out refractrometrically, which followed the method IFU No. 8.

5.3.3.5 pH value

The pH of the juice was measured through the pH electrode (Hanna, Italy) at 20°C (method IFU No. 11).

5.3.3.6 Turbidity before centrifugation

The measurement of the turbidity was performed through the turbidity equipment (Dr. Lange, Germany). The mean values were taken as the measured values of turbidity. The juice was diluted to 1:20 (juice: distilled water). The turbidity meter was calibrated by the solution with 400 TE/F. The level dilutions from this solution were obtained as the calibration curve.

5.3.3.7 Turbidity after centrifugation

The lemon juice was centrifuged at 370 x g for 10 min. Finally, the supernatant was diluted with distilled water to 1:20 respectively. The turbidity was measured as described above.

5.3.3.8 Colorimetry

The measurement of the juice colour was investigated by the colorimeter (Chroma-Meter CR-200, Minolta, Japan). The light value (L-value) and the tone (a and b value) were also determined. A-value means red when obtaining the positive value, and means green when negative value was obtained. B-value means yellow colour when obtaining the positive value and means blue when negative value was obtained.

5.3.3.9 Density

The related density of lemon juice was analyzed by density meter (Calculating Density Meter DMA 55, anton paar KG Graz, Austria).

5.3.3.10 Loss of cloud (clarification)

The loss of cloud was measured by the centrifugation of approx. 30 ml juice at 6000 x g for 15 min. The clarification of the supernatant was then measured in the spectrophotometer at 420 nm.

5.3.3.11 Statistical analysis

All experiments were run at least twice, and analyses of all samples were run triplicate and averaged. Statistical analyses were carried out using spreadsheet software. Significance of differences was defined at $P < 0.05$.

Part IV

Results and discussion

Chapter 6

Results and discussion

6.1 Discontinuous treatment

6.1.1 Temperature dependence of PE inactivation

The influence of thermal treatment was performed by the inactivation of lemon pectinesterase under standard condition of pH and reaction time. The non-linearity of the curves indicated that at least two fractions of pectinesterase existed in the lemon pulp with different thermostabilities. Apparently, the heat stable fraction accounted for ~10% of the total PE.

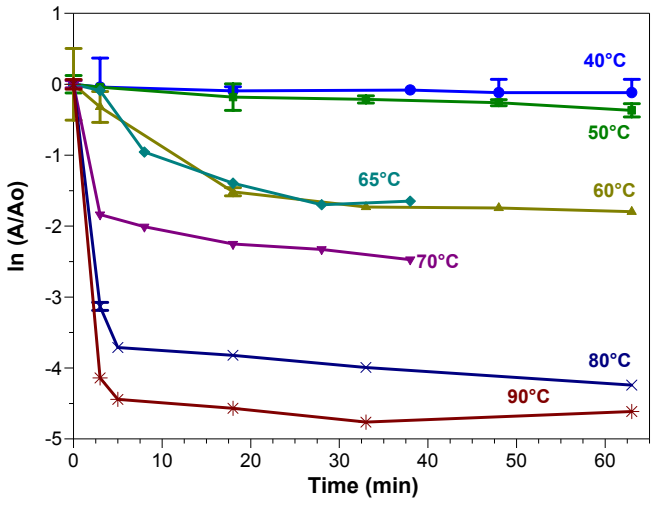


Figure 6.1: Heat inactivation of PE at various temperatures

In the temperature range between 40 and 50°C, the activities of PE dropped slightly during the period of 1 hour. However, this enzyme was increasingly inactivated at temperature above 60°C. Accordingly, the residual activity was approximately 4.36 % after heating at 80°C for 3 min and 1% after heating at 90°C for ~1 min. In Table 6.2, *D*-values were estimated from the plot in Figure 6.1, where *z*-value was obtained from *D*-value plot (Figure 6.2). E_a was derived from the Arrhenius equation of the rate constant (Table 6.2).

MacDonald et al. (1993) identified seven fractions of lemon pectinesterase, which considered being the heat sensitive and the heat stable fractions. Two major heat stable PE were found in peel and endocarp separately. These enzymes were completely inactivated at temperature above 88°C (MacDonald et al., 1993). In this study, the heat sensitive fractions were rapidly inactivated at 70°C. The *z*-value and E_a of heat treatment were calculated to be 16.2°C and 127.2 kJ/mol respectively. The *z*-values obtained earlier by some researchers for PE of orange, mandarin and grapefruit were 11, 11.4 and 5.2 °C, respectively (Eagerman & Rouse, 1976; Nath & Ranganna, 1977; Versteeg, 1979). In addition, the *z*-value of orange juice pulp PE were estimated to be 6.5 and 10.8°C for the sensitive and stable fractions (Wicker & Temelli, 1988).

6.1.2 Decimal reduction time of PE inactivation

The PE activity decreased with increasing time of exposure (Figure 6.1 and Appendix A.1). To evaluate the effect of treatment time, the decimal reduction time (*D*-value) was calculated and presented in Figure 6.2.

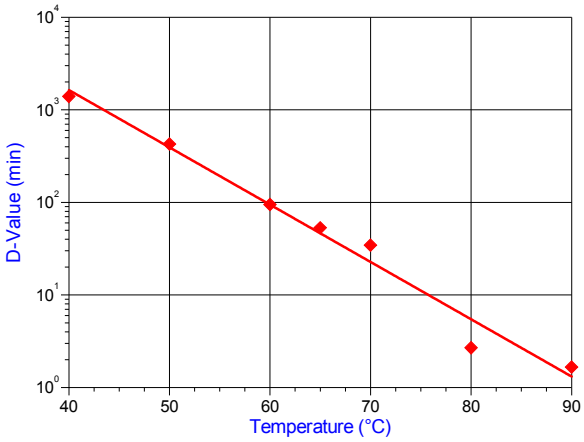


Figure 6.2: *D*-Value of PE inactivation at various temperatures

It has been found that the *D*-values at each temperature show significant differences (Table 6.2). The *D*-value at 90°C was 1.66 min. Eagerman and Rouse (1976) also reported processing orange juice at 90°C for *D*-value of 30 sec was enough to stabilize the cloud. Versteeg (1978) demonstrated a *D*-value of 22 sec at 90°C is necessary to inactivate the heat stable PE isozyme. In this study, *D*-values for PE inactivation ranged from 1.66 min at 90°C to 34.5 min at 70°C (Table 6.2). However, the *D*-value of the experiment at 90°C was greater than the values from Eagerman and Rouse (1976), and Versteeg (1978) due to the comprising of the time to inactivate both of heat labile and heat resistance portion of the enzyme. Therefore, no real *D*-values could be obtained in this experiment.

6.1.3 Effect of pH on PE inactivation

The pH of PE in citric acid and Sodium Citrate buffer was adjusted at 2.5, 3.5, 5.5 and 7.5. The activity of the PE was consequently evaluated.

Table 6.1: Effect of pH on the PE activity (at 30°C, 1 min)

pH of PE in citric acid and Sodium Citrate buffer	Activity (unit/ml)
2.5	0.55
3.5	2.10
5.5	2.30
7.5	2.50

It has been found that the pH has an influence on the PE activity, which greatly reduced at low pH of 2.5. This result was in agreement with many researchers (Atkins and Rouse, 1953; Eagerman and Rouse, 1976). They have demonstrated that PE inactivation rate was dependent on the pH of the juice, with increased inactivation rates occurring at the lower pH values. Versteeg et al (1978) and Marshall et al. (1985) also reported that the enzyme was very stable at higher pH value specifically at 7.

6.1.4 Discussion on thermosonication inactivation of lemon PE

Pectinesterase was not inactivated even after 1 hour of exposure to the cavitating 20 kHz ultrasound at room temperature at pH 7. Therefore, it was suggested to combine the ultrasonic treatment with temperature (thermosonication) to increase the inactivation rate. The experiments were set up by applying ultrasound 20 kHz with various temperatures for different periods. The temperature and the flow rate of water circulating in the water bath were set. Therefore, the temperature of the PE suspension remained constant during the experiment. Actually, the temperature varied at ±0.3°C with respect to the average temperature. In all experiments, the PE deactivation due to ultrasound treatment was non-linear according to the presence of more than one fraction of PE in the pulp. From the comparison of the results depicted in the Figure 6.3, it reveals that the combined treatment has the additive effects.

Moreover, the inactivation effect of the thermosonication above 50°C was much greater than the inactivition of heat treatment at the same temperature.

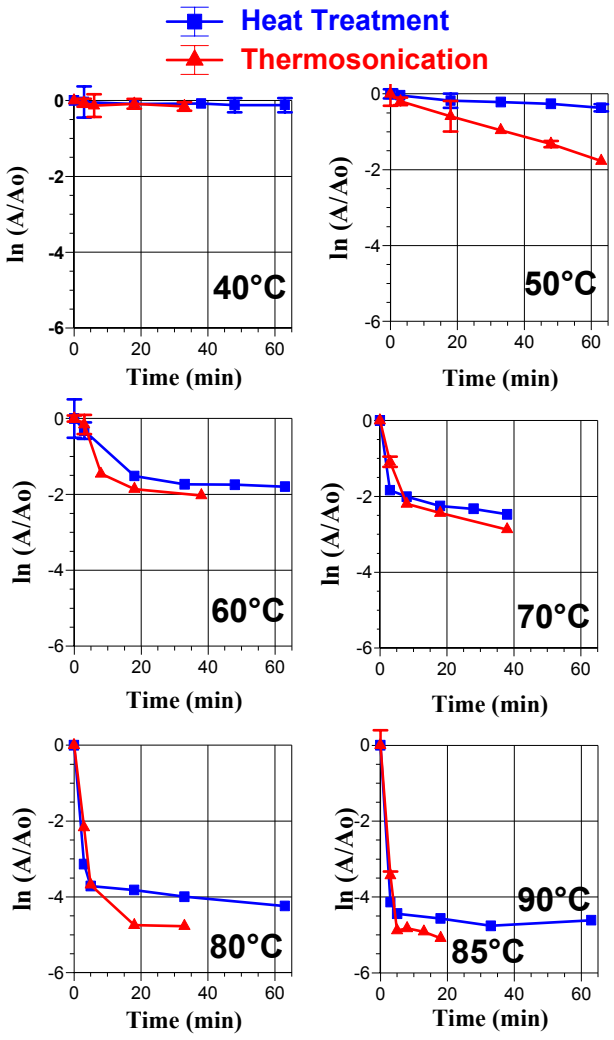


Figure 6.3: Heat and thermosonication inactivation of lemon PE

The *D*-values of PE treated by thermosonication were also less than the *D*-values obtained from heat treatment at the same temperature. The heat stable PE has been found to be sensitive to the ultrasonic waves. *z*-value and E_a of thermosonication treatment were obtained to be 18.8°C and 120.5 kJ/mol respectively. In this case, the synergistic effect does not alter the *z*-value and the activation energy. It has been shown that there are no significant differences between the values of *z* and E_a obtained from the heat and the combined treatment (Table 6.2).

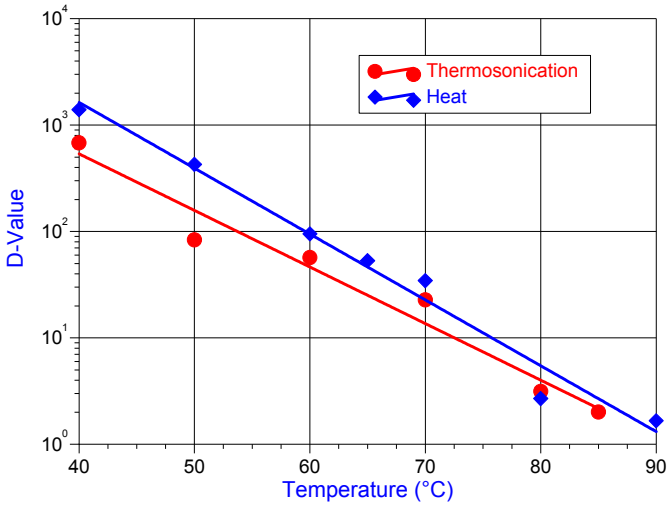


Figure 6.4: *D*-value of PE inactivation by heat and thermosonication

Table 6.2: *D*-values of heat and thermosonication PE inactivation

Temperature (°C)	*D*-value of heat treatment (min)	*D*-value of thermosonication treatment (min)
40	1396.56	681.40[b]
50	426.42	83.38[b]
60	94.82	56.92[b]
65	53.12	-
70	34.47	22.79[b]
80	2.69	3.14[b]
85	-	2.011[b]
90	1.66	-
***z*-value (°C)**	16.16	18.80[a]
***E_a* (kJ/mol)**	127.18	120.54[a]

a = no significant difference between heat and thermosonication treatment
b = significant difference between heat and thermonsonication treatment ($P < 0.05$)

6.2 Lab scale continuous ultrasonication

6.2.1 Effect of treatment temperature on lemon PE inactivation

The activity of PE was almost linear (first-order reaction). This phenomenon was in accordance with normal enzyme behaviour and heat-activation of chemical reactions according to the Arrhenius law. The results of these experiments were plotted in Figure 6.5.

It is shown that the temperature below 60°C has no significant inactivation effect on the enzyme. However, the inactivation started above 70°C and dramatically increased at 90°C.

From this plot, a *z*-value of 16.12°C for the heat inactivation reaction was calculated. Accordingly, from the Arrhenius inactivation rate constant (derived from *D*-values; Table 6.3), the activation energies E_a of 156.655 kJ/mol was computed. Vercet et al. (1997) presented the *z*-value of 5.1 °C and E_a of 435 kJ/mol for the thermoresistant orange PE, which was seemingly different from the lemon PE in this study.

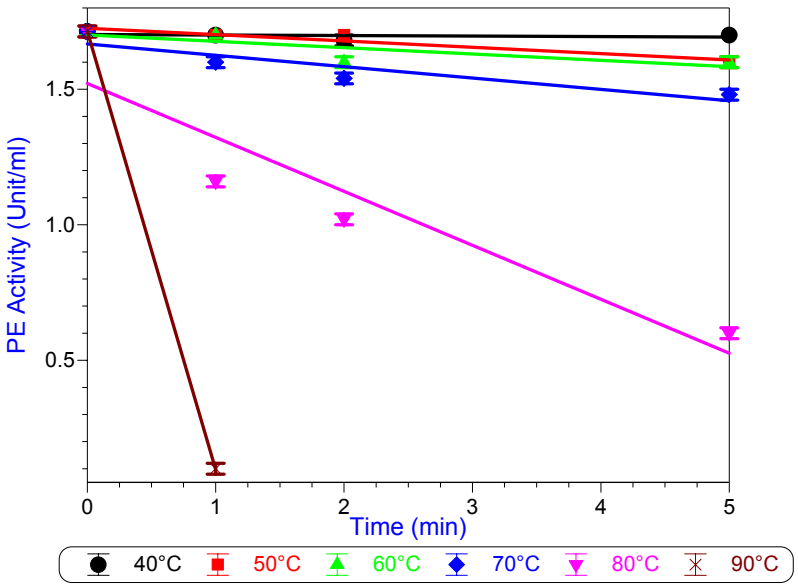


Figure 6.5: Thermo-stable PE inactivation at various temperatures

Table 6.3: *D*-values and inactivation rate constant of heat treatment

Temperature (°C)	*D*-value (min)	k (min^{-1})
40	2071.01	1.112×10^{-3}
50	163.32	0.01
60	163.17	0.01
70	86.90	0.05
80	11.71	0.17
90	0.52	4.45

6.2.2 Effect of pressure on lemon PE inactivation

Table 6.4 shows the residual activity at various pressures and temperatures for PE. At the temperature below 70 °C, it is no remarkable addition effect of pressure on the inactivation reaction. Therefore, the application of moderate pressure has only small-enhanced effect in terms of lemon PE inactivation.

Table 6.4: Temperature and pressure inactivation of thermoresistant PE (see Appendix A.4, A.5, A.6)

Temperature (°C)	Time (min)	PE activity under 100 kPa (unit/ml)	PE activity under 200 kPa (unit/ml)	PE activity under 300 kPa (unit/ml)
40	0	1.92±0.04	1.90±0.00	1.70±0.00
	2	1.87±0.06	1.72±0.03	1.61±0.05
	5	1.80±0.00	1.70±0.00	1.49±0.04
50	0	1.92±0.04	1.90±0.00	1.70±0.00
	2	1.67±0.05	1.61±0.05	1.40±0.00
	5	1.40±0.00	1.30±0.00	1.09±0.03
60	0	1.97±0.06	1.90±0.00	1.70±0.00
	1	1.72±0.03	1.70±0.00	1.49±0.04
	2	–	1.52±0.00	1.28±0.00
	5	1.09±0.03	1.00±0.00	–
70	0	1.90±0.00	1.90±0.00	1.84±0.00
	2	1.40±0.00	1.43±0.04	1.20±0.02
	5	0.70±0.00	0.58±0.00	–
80	0	1.97±0.06	1.90±0.00	1.70±0.00
	2	1.40±0.00	–	1.12±0.00
	5	0.58±0.00	0.43±0.01	–

Pressure dependence was determined according to the Eyring equation from log-linear plots of the inactivation rate constant vs. time at various level of pressure. Activation volumes were presented in Table 6.5. The activation volumes were negative at all studied temperatures, indicating that inactivation of PE was enhanced by an increase in pressure.

At higher temperature (70-80°C), the pressure level required for inactivation could be reduced. For temperatures above 70°C, increase in pressure slightly enhanced the inactivation reaction. Changes in hydrostatic pressure have been shown to alter the rates of some sonochemical reactions by an order of magnitude (Lopez et al., 1994).

It was difficult to keep the pressure steady during the experiment since the implosion of the bubble caused the pressure changes in the medium. Therefore, it is necessary to control the outlet valve constantly in order to keep the pressure uniform in the overall process.

Table 6.5: *D*-values and V_a of the combination treatment of temperature and pressure

Temperature (°C)	*D*-value at 100 kPa (min)	*D*-value at 200 kPa (min)	*D*-value at 300 kPa (min)	V_a (cm^3/mol)
40	71.82	45.01	38.03[b]	-8271.27[a]
50	15.07	13.84	10.08[b]	-4281.19[a]
60	9.29	7.87	7.11[b]	-3631.99[a]
70	4.95	4.12	4.78[b]	-498.80[a]
80	4.05	3.44	2.97[b]	-4568.82[a]

a = no significant difference between heat and thermosonication treatment
b = significant difference between heat and thermonsonication treatment ($P < 0.05$)

6.2.3 Effect of treatment time on lemon PE inactivation

The calculated *D*-value at 90°C of thermoresistant PE was 30.02 sec. This obtained value was similar to the result of Eagerman and Rouse (1976), who reported that a *D*-value of 30 sec was enough to stabilize the citrus juice from cloud loss. However, Versteeg's *D*-value of 22 sec at 90°C for the heat stable PE isozyme was lower than the *D*-value obtained from this experiment.

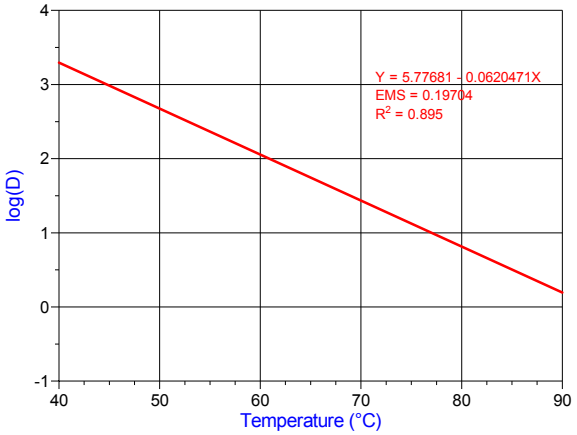


Figure 6.6: log (D) vs. temperature plot of PE heat treatment

The plot of log(*D)* vs. temperature (Figure 6.6) was apparently linear, which was shown to follow the Arrhenius kinetic behaviour.

6.2.4 Discussion on manothermosonication inactivation of lemon PE

The inactivation of PE by manothermosonication (MTS) was investigated in the pressure range 100 to 300 kPa at temperature varying between 40 and 80°C, and ultrasound 20 kHz. Duration of each experiment was 5 min (Figure 6.7). The temperature profiles at 100-300 kPa were presented in the Appendix B.1 to B.3.

According to all studies, the inactivation could be described by a first order kinetic model. The decimal reduction values for the combination treatment were presented in Table 6.6, showing the synergistic effect to increase with increasing pressure and temperature.

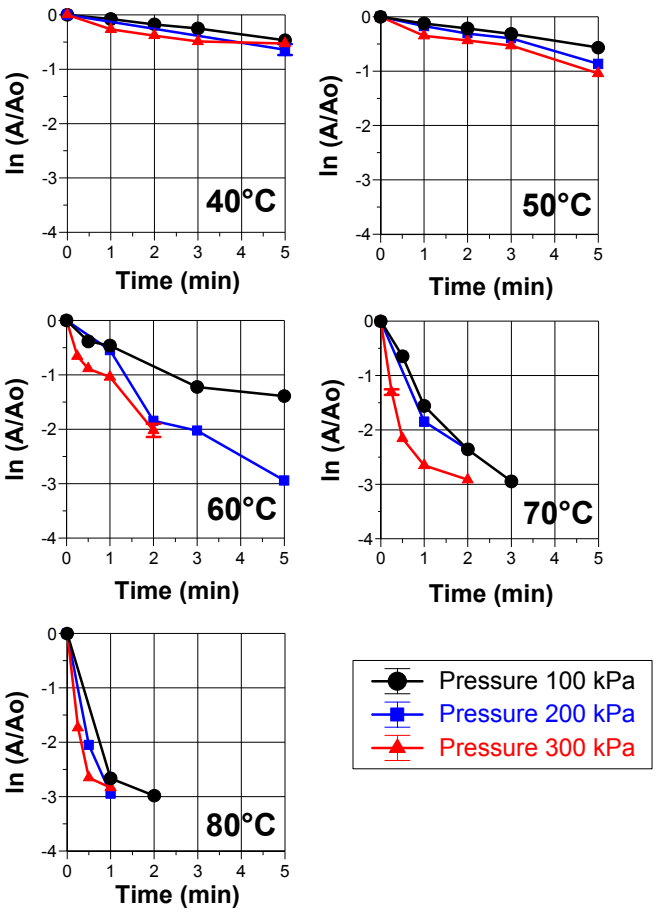


Figure 6.7: Manothermosonication inactivation of thermoresistant PE

Table 6.6: *D*-values and V_a of manothermosonication treatment

Temperature (°C)	*D*-value at 100 kPa (min)	*D*-value at 200 kPa (min)	*D*-value at 300 kPa (min)	V_a (cm^3/mol)
40	10.68	7.79	10.00	-845.44
50	8.98	5.99	5.23	-7282.05
60	3.66	1.67	1.18	-16408.29
70	1.02	0.85	0.80	-3433.22
80	0.67	0.34	0.38	-8288.40

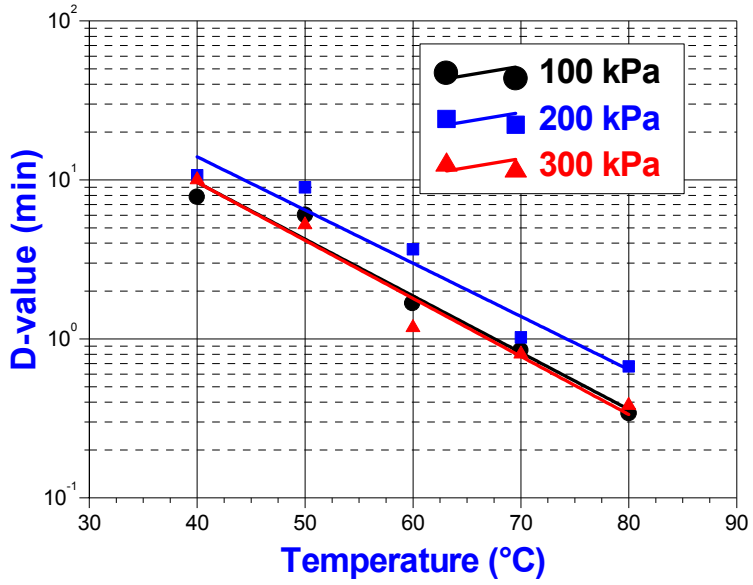


Figure 6.8: *D*-value of manothermosonication inactivation

As to temperature dependence of the decimal reduction time or *D*-value, activation energies at different constant pressure levels were derived (Table 6.7). These results were in agreement with the results reported by Vercet (1999), who observed higher reaction rate constant and lower *D*-value for MTS inactivation of orange PE in comparison with its heat inactivation. They also demonstrate that *z*-value for MTS inactivation was higher than that obtained for its heat inactivation.

For all studied temperature, increase in pressure enhanced the inactivation reaction rate and reduced the decimal reduction time. However, at higher temperature (70-80°C), the pressure level required for inactivation could be reduced. In the Table 6.7, it is shown that

the synergy did not alter the *z*-value, which was estimated to be 28°C. The phenomenon that the *z*-value of the PE inactivation remained unchanged when ultrasonic waves were simultaneously applied can be implied that, the temperature dependence of the reactions responsible for the ultrasonic waves destructive effects was equal to the temperature dependence of the reaction responsible for its thermal inactivation.

Table 6.7: *z*-values and E_a of manothermosonication treatment

Pressure (kPa)	*z*-value (°C)	E_a (kJ/mol)
100	28.01	63.53
200	29.88	71.91
300	27.40	74.97

The decrease in MTS enzyme inactivation efficiency as temperature increased seems to be well known (Lopez and Burgos, 1995; Vercet et al., 1997). It appears to be mainly due to the decrease of the collapse intensity, because of the elevation of the water vapour pressure inside the bubble, as temperature increases (Vercet et al., 1997). It has been revealed that the effects of heat and ultrasonic waves in enzyme inactivation combine synergistically. This is actually implied from the result that the inactivation rate of combined method is greater than the sum of the rate of inactivation by ultrasound at room temperature and the rate of inactivation by simple heating. It is also well known that ultrasonic wave enhances the fluid-to-particle convective heat transfer (Kuldiloke, 1995). From these bases, the lemon PE inactivation was achieved. The *D*-value of inactivation of the thermoresistant PE fraction from lemon juice is greatly reduced by ultrasonic irradiation while heating at the temperature below 80°C. It has been found that MTS could dramatically reduce the intensity (time and/or temperature) of the heat treatments used for lemon juice stabilization. Temperatures between 65 and 70°C were likely to be the most appropriate treatment for this purpose, since the citric juice microflora is not specifically thermo-resistant. A few seconds at this temperature would be enough for its destruction (Vercet, 1999). Moreover, it has been suggested that the ultrasonic waves enhance microbial heat destruction (Ordonez et al., 1987). However, the application of MTS into the lemon juice industry will require further information of its effects on nutrients and the

sensorial and rheological properties of the juice. The suitable industrial equipment should also be determined.

In order to analyze the mechanisms of the ultrasonic PE inactivation, there are several assumptions that can be considered. When ultrasound wave is applied to a liquid, it promotes the acoustic cavitation. The bubbles in the liquid are changed in shape and size continuously. This makes an acoustic stream adjacent to the bubble, which often results in severe shear stresses. The stresses can promote enzyme denaturation. The cavitational collapse of the bubble in transient cavitation also generates extremely high local pressures and temperatures, which can make the in vicinity enzyme destructive. Sonication also promotes chemical reaction involving H• and OH• free radicals formed by the decomposition of water inside the oscillating bubbles (Lopez et al., 1994). These free radicals could be scavenged by some amino acid residues of the enzymes participating in structure stability, substrate binding, or catalytic functions. These mechanisms are responsible for the synergistic effect observed in MTS enzyme inactivation. One cannot deduce any of them from the experimental results in the study. These mechanisms are also dependence on many variables such as liquid temperature, ambient pressure, and ultrasonic intensity. The ultrasonic intensity, itself, reflects the effect of these parameters on bubble dynamics, number of bubbles, and pressure and temperature inside the collapsing cavitation bubbles. Therefore, it can be implied that more than one mechanism is operative. Moreover, the inactivation of some enzymes at low temperatures by long time exposures to an ultrasonic field has been known for more than 30 years (El'piner; 1964). This low temperature inactivation has been generally attributed either to the splitting of low molecular weight polypeptides or individual amino acids or, more frequently, to oxidative mechanisms (Lopez et al., 1994).

6.2.5 The inactivation of tomato polyphenoloxidase, peroxidase, pectinesterase, and polygalacturonase

6.2.5.1 Polyphenoloxidase

Figure 6.9 presents the effect of polyphenoloxidase (PPO) heat inactivation at various temperatures. The temperature below 60°C has slightly effect on the inactivation. However, at temperature above 70°C, the inactivation was significantly increased and almost completed. The inactivation of tomato PPO showed the biphasis behaviour, which indicates that there was the presence of different heat stability of PPO. Jolly and Nelson (1969) and Strotkamp et al. (1974) also reported the PPO isozyme behaviour in the mushroom.

Table 6.8: The *D*-value (min) of thermal treatment and the combination treatment of heat, pressure and ultrasound on PPO

	Ambient pressure		400 kPa	
Treatment temperature (°C)	**Without ultrasound**	**With ultrasound**	**Without ultrasound**	**With ultrasound**
40	23.72±10.21	15.99±6.01	25.26±9.7	12.44±2.29
50	17.81±6.38	13.32±6.20	16.62±7.07	9.60±3.01
60	14.73±9.97	11.86±5.55	16.04±5.43	8.98±2.34
70	5.41±3.21	6.81±0.90	5.82±1.82	3.70±1.35
80	2.25±1.20	1.98±1.04	1.49±0.96	1.30±0.91
90	2.22±1.42	0.87±0.45	0.96±0.56	0.89±0.56

The combination of heat and ultrasound increased the inactivation of PPO at treatment temperature 40°C-70°C. The *D*-values of the thermosonication (40°C - 60°C) are 20 - 25% lower than the values obtained from the heat treatment (Table 6.8).

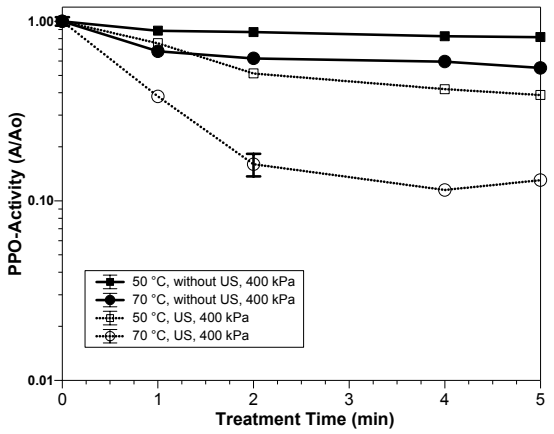


Figure 6.9: The influence of temperature with and without ultrasound treatment on PPO activity

Figure 6.9 shows the effect of the manothermosonication at various temperatures and pressure of 400 kPa. From this figure, it is clear that the increasing of manothermosonication inactivation occurred during the increasing of temperature. The calculated *D*-values of the manothermosonication treatment are much lower than only heat treatment at the same conditions (Table 6.8).

6.2.5.2 Peroxidase

Table 6.9 presents the effect of heat treatment, thermosonication, and manothermosonication on the inactivation of peroxidase (POD) respectively. It is apparently shows that the inactivation of tomato POD follows the biphasis behaviour. Lopez et al. (1994) also observed heat and MTS inactivation effect of POD from horseradish. It has been found that the MTS inactivation of POD follows the first-order kinetic (Lopez, 1994).

In this experiment, the POD inactivation shows the biphasis behaviour according to the presence of different heat-stability isozymes. The investigation on POD isozyme has not been made in this experiment. Maragoni et al. (1989) has determined three different POD isozymes in tomato.

The combination of heat and ultrasound cause the synergistic effect on the POD inactivation. Accordingly, the increased temperature during the manothermosonication treatment also significantly increases this synergistic effect (Figure 6.10).

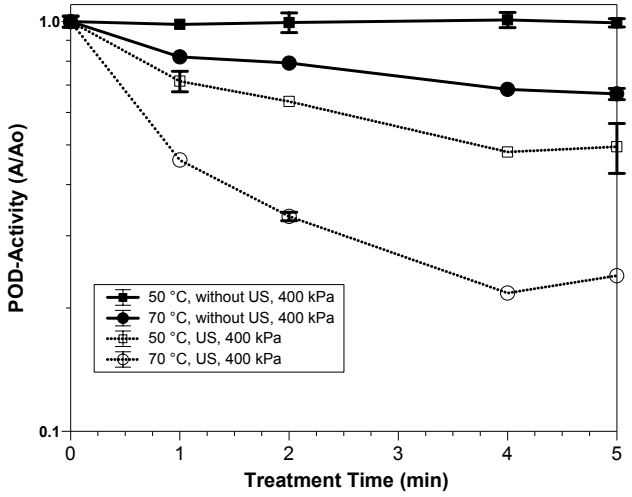


Figure 6.10: The influence of temperature with and without ultrasound treatment on POD activity

The *D*-values of heat treatment range from 37 min at 40°C to 8 min at 70°C, and the *D*-values of the manothermosonication range from 11 min to 5 min at the same conditions and pressure 400 kPa (Table 6.9).

Genaro et al. (1999) have observed the D_{80} of horseradish POD (type VI) of 56 min of heat treatment and 10 min by the combination of heat and ultrasound. They also determined that the *D*-value depended on the ultrasound power, the geometry of the sonotrode and the volume of the treatment medium.

Table 6.9: The *D*-value (min) of thermal treatment and the combination treatment of heat, pressure and ultrasound on POD

	Ambient pressure		400 kPa	
Treatment temperature (°C)	**Without ultrasound**	**With ultrasound**	**Without ultrasound**	**With ultrasound**
40	37.91±6.34	29.99±6.50	43.28±10.27	11.02±3.43
50	36.18±12.73	21.13±8.74	34.79±11.34	9.39±2.68
60	21.08±13.49	12.63±7.28	26.57±6.77	7.39±2.31
70	8.02±4.62	7.33±2.65	8.56±3.61	5.08±2.17
80	2.51±1.08	2.25±0.60	1.55±0.80	1.28±1.02
90	1.66±1.27	0.77±0.34	0.95±0.50	0.82±0.52

6.2.5.3 Pectinesterase

Similarily to the PPO and POD, the inactivation of tomato pectinesterase (PE) shows the biphasis kinetic behaviour (Figure 6.11). Lartta et al. (1995) have extracted three PE isozymes from tomato with various heat stability. The thermo-stable fraction of PE is approximately 70% of the total amount of the enzyme in tomato. Therefore, the complete inactivation of this enzyme is essential for the protection of cloud loss (Lartta et al., 1995).

Van den Broeck et al. (2000) determined the biphasis inactivation of orange PE. De Sio et al. (1995) also investigated the heat stability of tomato PE from five different sources at 73-88°C. The *z*-values of the PE inactivation show the biphasis behaviour, which dramatically increased at 78-88°C. With this increased *z*-values at higher temperature, it

can be implied that there was the presence of the heat-stable fraction of PE. The *D*-values of heat treatment in this experiment vary from 63 min at 40°C to 5 min at 70°C (Table 6.10).

The combination of ultrasound and heat causes the significant reduction of the PE activity (approximately 10% of its initial value at 70°C for 4 min), comparing to the heat inactivation at the same condition. Moreover, the increased pressure of 400 kPa in the manothermosonication treatment achieves more than 90% PE inactivation at 70°C for 4 min. The reduction of *D*-value (55 min at 50°C by heat treatment and 24.5 min by manothermosonication treatment at the same condition) shows the positive result of the combination method of ultrasound, heat and pressure.

Table 6.10: The *D*-value (min) of thermal treatment and the combination treatment of heat, pressure and ultrasound on PE

	Ambient pressure		400 kPa	
Treatment temperature (°C)	**Without ultrasound**	**With ultrasound**	**Without ultrasound**	**With ultrasound**
40	63.37±25.69	62.53±18.28	74.65±29.05	29.62±5.53
50	51.83±19.24	39.53±14.25	55.12±20.71	24.43±4.55
60	12.81±7.99	22.49±10.62	14.38±7.20	19.138±7.65
70	4.81±1.35	8.05±1.84	4.98±0.67	3.36±1.02
80	1.09±0.14	1.47±0.48	0.99±0.51	0.57±0.39
90	–	0.26±0.00	0.32±0.08	0.29±0.06

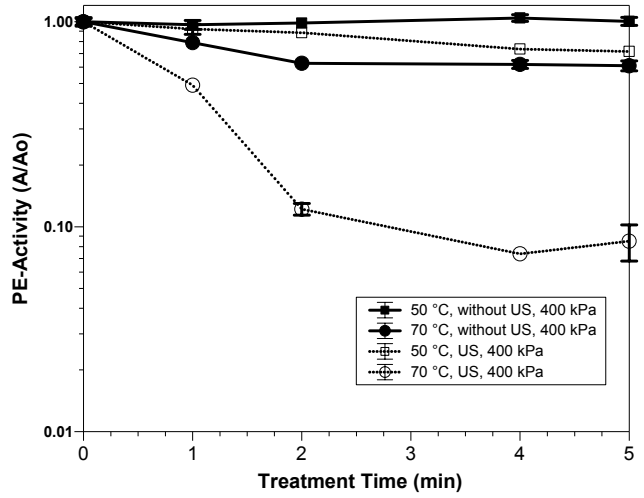


Figure 6.11: The influence of temperature with and without ultrasound treatment on PE activity

6.2.5.4 Polygalacturonase

Similar to the PE inactivation, polygalcturonase (PG) was inactivated at the temperature above 70°C for 5 min (Figure 6.12). Table 6.11 presents the *D*-value obtained from the heat treatment, thermosonication and manothermosonication treatment. The combination of heat (40-60°C) and ultrasound (at 400 kPa) decreased the *D*-value for about 2-3 times lower than heat treatment only. Lopez et al. (1997) defined two PG isozymes from tomato (PGI and PGII). Both fractions performed the first-order inactivation kinetic with *D*-values of 2.14 min at 64°C for PGI and 0.24 min at 73°C for PGII. The PGI was more heat-stable enzyme. Differently from Lopez et al. (1997), the PG isozymes were not isolated in this present work. Therefore, the inactivation was observed to follow the biphasis behaviour due to the presence of different thermostability of enzyme (Figure 6.12). The *D*-value was ~13 min at 60°C and 4.5 min at 70°C (table 6.11). However, the *D*-value obtained from the manothermosonication treatment was ~5.6 min at 60°C and 2.9 min at 70°C (Table 6.11).

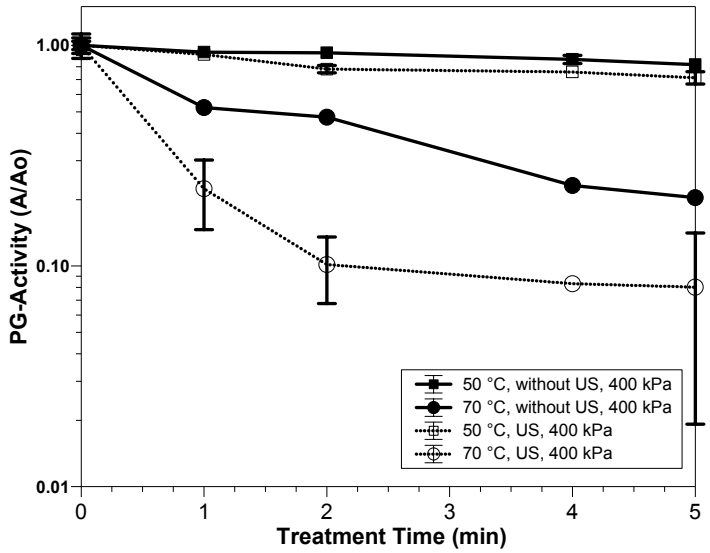


Figure 6.12: The influence of temperature with and without ultrasound treatment on PG activity

Table 6.11: The *D*-value of thermal treatment and the combination treatment of heat, pressure and ultrasound on PG

	Ambient pressure		400 kPa	
Treatment temperature (°C)	**Without ultrasound**	**With ultrasound**	**Without ultrasound**	**With ultrasound**
40	60.81±20.86	54.21±18.54	58.00±17.29	31.57±5.54
50	41.33±18.63	25.25±7.21	40.96±18.27	14.77±4.74
60	12.74±4.61	22.96±5.07	14.00±2.97	5.63±2.27
70	4.34±0.33	4.87±0.16	4.40±0.78	2.90±1.32
80	2.16±0.89	2.23±1.20	2.32±1.61	1.28±0.80
90	0.79±0.00	0.61±0.00	1.32±0.80	1.28±0.74

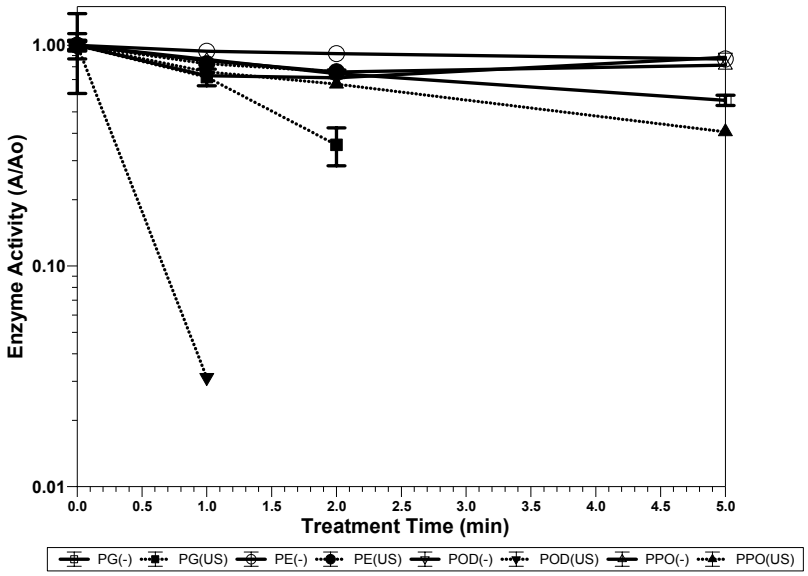
6.2.5.5 Influence of the combination treatment of ultrasound and CO_2 on PE, PPO, POD and PG

The addition of CO_2 at 400 kPa during the ultrasonication at 60°C significantly induced the inactivation of PPO, PE, POD and PG, comparing to the ultrasound treatment without CO_2 at the same conditions (Figure 6.14). It has been reported previously that the CO_2 gas enhances the heat inactivation of enzyme (Tedjo et al., 2000; Chen et al., 1993).

The influence of the combination treatment of ultrasound and CO_2 on the inactivation is not well known. The implosion and the effect of ultrasound were reduced with the increased gas amount (Mason, 1988). However, the *z*-value of this effect was enhanced. The CO_2 gas can reduce the pH of the treatment medium and effect the enzyme inactivation (Chen et al, 1993). Lu and Whitaker (1974) has determined that the release of Hemin- group from POD was depended on the pH value especially lower than 5.

The cavitation induces the vapour compression and heat up the surround liquid suddenly. This generates the sudden hot spots in the medium. Accordingly, this implosion is probably favour to the condition of the super-critical CO_2 ($\geq$ 7380 kPa, $\geq$ 31°C). The influence of the super-critical CO_2 on enzyme activity has been reported by many researchers (Tanaguchi et al., 1987; Randolph et al., 1985; Kasche et al. 1988 and

Hammund et al., 1985). Tedjo et al. (2000) determined an increased peroxidase and lipoxygenase inactivation by the super-critical CO_2 (100 - 600 kPa) at moderate temperature (35-50°C) in comparison to the thermal treatment at the same condition at atm. pressure. The exact inactivation mechanism of the combined treatment of ultrasound with CO_2 is necessary to be investigated further.



US = Ultrasonic treatment with CO_2 (treatment at 400 kPa, 60°C)
- = without ultrasonic treatment, with CO_2 (treatment at 400 kPa, 60°C)

Figure 6.13: The effect of the combination treatment of ultrasound with CO_2 at 400 kPa, 60°C on PPO, POD, PE and PG activity

6.3 Pilot plant scale of ultrasonic treatment of real juice

6.3.1 Effect of manothermosonication on PE inactivation of fresh lemon juice

The inactivation percentage of fresh lemon PE was performed in Figure 6.14. The temperature profile was presented in Appendix B.4, as well as the exact time that the samples were taken. The enzyme was hardly inactivated, however the power of the ultrasound has great impact and could improve the inactivation.

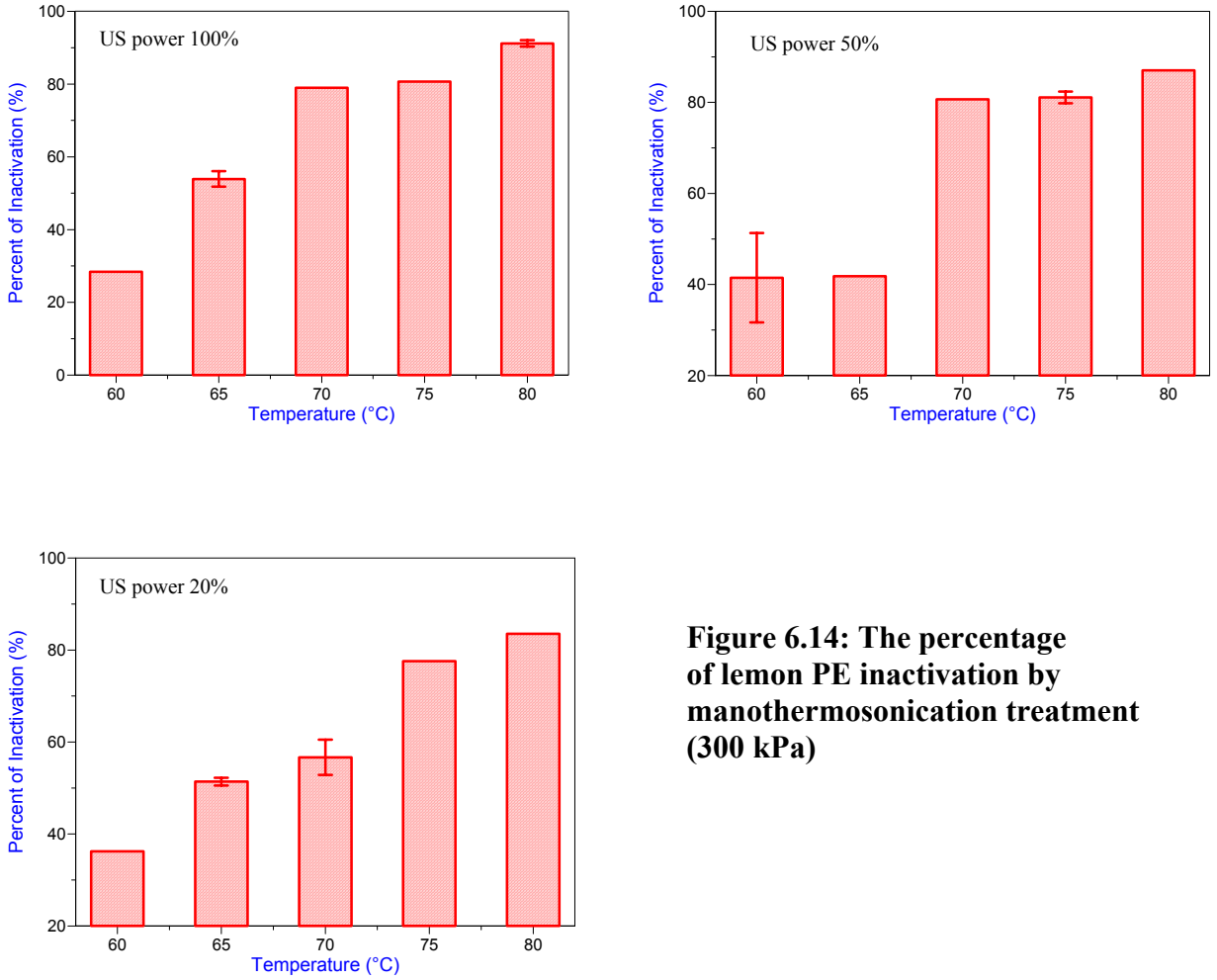


Figure 6.14: The percentage of lemon PE inactivation by manothermosonication treatment (300 kPa)

It has been found that the percentage of enzyme inactivation was increased as the temperature increased. At 80°C and 300 kPa, PE enzyme was inactivated by 83.5% with operating power of 20%, 87% with the power of 50% and 91.2% with the power of 100%.

To determine the effect of MTS enzyme destruction efficiency with the sonication power, the residual activity of PE has been plotted vs. the sonication power in Figure 6.15. It is well known that increased intensity of ultrasound irradiation increases the sonochemical reaction rate, the greater the amplitude, the higher efficiency (Suslick, 1988).

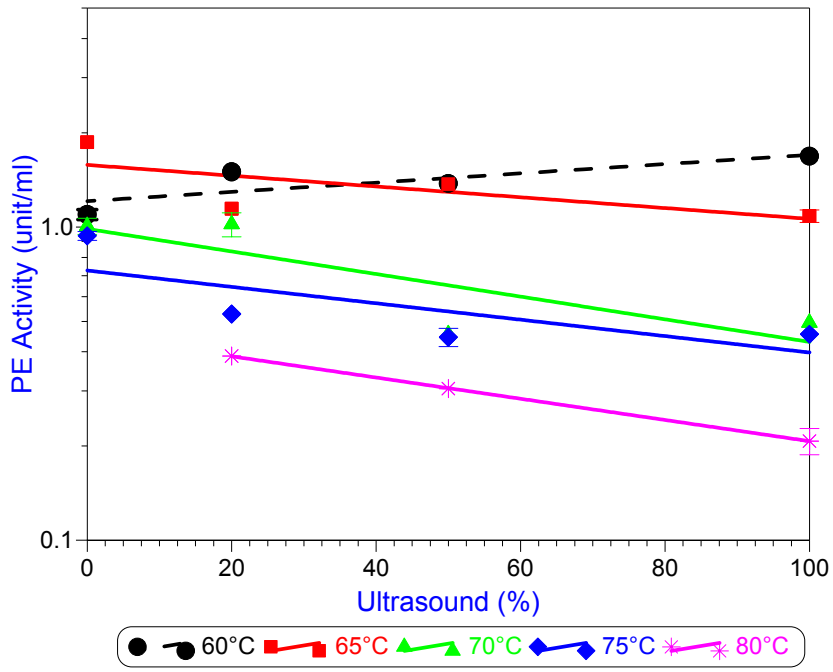


Figure 6.15: The effect of different level of ultrasound on the manothermosonication inactivation of thermoresistant lemon PE

As shown in Figure 6.15, the power of the ultrasound generally followed the suggestions of Suslick (1988) in the reduction of the enzymes. However, it was not true for moderate temperature treatment, such as 60°C. The activity of PE was slightly increased when higher power of ultrasound was applied to the enzyme solution. This phenomenon can be assumed that, as the power of ultrasound increases with mild heating (< 65°C), the enzyme can be increasingly activated. The ultrasonic inactivation reaction is obviously temperature dependence.

However, it is difficult to determine the certain effects of ultrasound in fresh lemon juice due to many components in the juice itself. Some of them may protect the PE inactivation, such as pectin and sugar. This assumption has also been investigated by Vercet et al. (1999). They emphasized that the most important factor in determining the PE inactivation seemed to be pectin. In addition, substrate-mediated protection of several other

enzymes against heat and other activating agents has been described previously (Klibanov, 1983). PE in fresh lemon juice was more difficult to inactivate than PE in distilled water, even though the pH in the fresh juice was comparatively low (pH 2.45). This is due to the nature PE in fresh lemon juice is strongly bound to pectin. This kind of physicochemical state seems to require the inactivation energy.

Nevertheless, the MTS inactivation of PE in fresh lemon juice can be a promising method in juice preservation, since the enzyme inactivation level can be met at the temperature under 100°C. However, it is also necessary to evaluate the composition properties of the treated juice as well as the microbial contamination and shelf-life.

6.3.2 Effect of manothermosonication on lemon juice quality

6.3.2.1 Turbidity

No large effect from the storage for the turbidity after 3-5 storage days at 4°C comparing to the fresh squeezed juice could be observed. It is also implied that temperature at 4°C can be considered as the suitable storage environment according to the turbidity (Appendix A.11).

The treated juice (the combination process and heat treatment) had higher turbidity than untreated juice after centrifugation at 370 x g for 10 min. The treated juice from the combination process was also shown to have higher turbidity than the heat-treated juice.

The centrifuged juice has clearly shown the change of the turbidity of stored juice. The untreated juice had less turbidity than the heat and MTS treated juice. Accordingly, the thermal treated juice had lower turbidity values than the treated juice from the combination processes. The ultrasound treated juice showed no large difference of the turbidity after the storage (Appendix A.12).

6.3.2.2 Brix value

No difference of Brix value was found during the storage time (Appendix A.11, A.12).

6.3.2.3 Density

The storage caused no change of the density of the juice (Appendix A.11, A.12).

6.3.2.4 pH

There was no difference of pH between the different treatments (the combination process and the single treatment of heat). The pH value of the treated juice was also the same as the untreated juice (Appendix A.11, A.12).

A slight increase of pH has been observed for the treated and untreated juice during the storage. This could be discussed as the general changes of chemical or biochemical substances in the juice itself, and/or the difficulty of pH-meter set up, which causes the pH shifted.

6.3.2.5 Loss of cloud

A clear effect of the combination treatment was the loss of cloud. After centrifugation at 6000g, the loss of cloud was measured. The MTS treated juice showed greater value of cloud comparing to the untreated juice. This could imply that pectin still remained as the form of suspended solid, which made the juice more fresh-looked. This rheological property is significantly required in terms of industrial satisfaction. The MTS treatment has produced the results that favour the requirement and, therefore, could be one of the promising potentials in the lemon juice industry.

Figure 6.16 presents the untreated and MTS treated juice after the 18 days at 4°C. It is clearly shown that almost complete sediment occurred in the untreated juice. In contrary, the MTS treated juice (at 80°C, ultrasounic amplitude 100%) still contained the colloidal look as similar as in the fresh-squeezed juice.

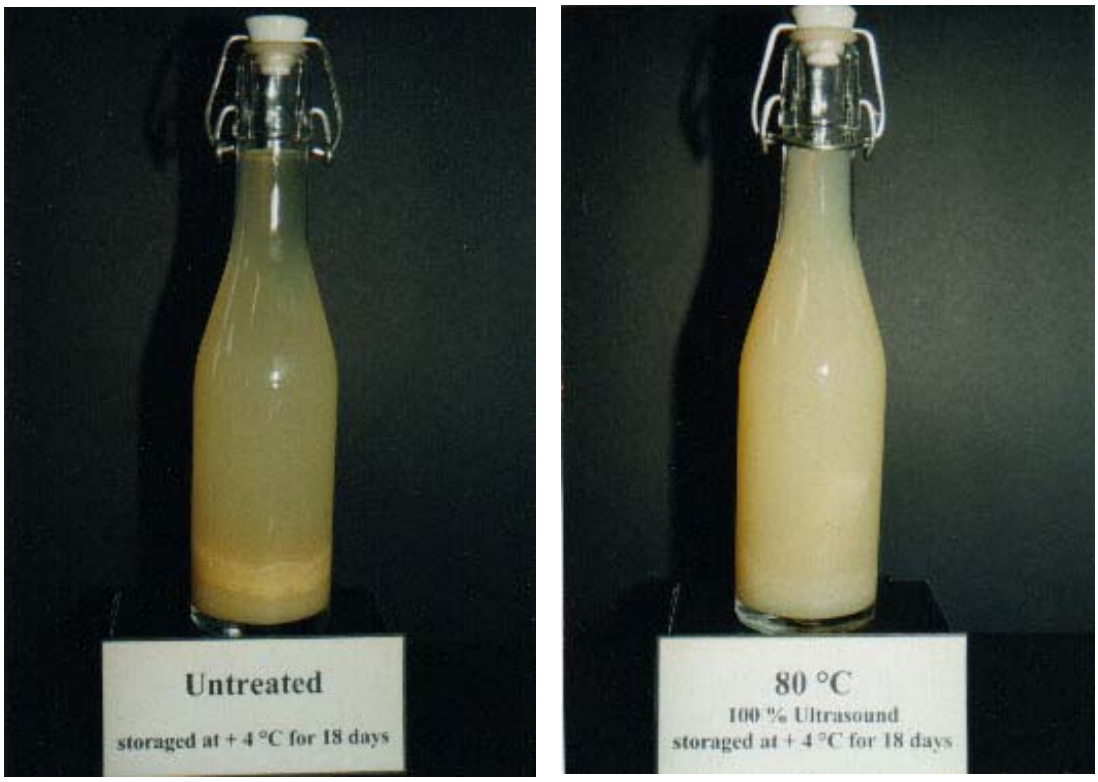


Figure 6.16: The untreated and treated lemon juices after 18 days (stored at 4°C)

6.3.2.6 Microbiology

The microbial count of the fresh lemon juice is approximately 10^3 cells/ml, which can be slightly increased during the storage time (Data not shown). The treated juice (from the combination processes and the single process of heat treatment) showed no viable counts. After storage for 18 days, no increase of the viable cell culture was observed.

Raso et al. (1998) have studied on the MTS lethality of food-borne pathogen *Yersinia enterocolitica*. Authors have reported that the microbial destruction was seemingly due to two different mechanisms acting independently; one of heat and the other of MS. The lethal effect of MTS treatment would be the results of MS plus heat independently. Moreover, these mechanisms depended on the treatment temperature.

6.3.2.7 Ascorbic acid

One of the important nutrition is ascorbic acid. Pasteurization has great effect on the reduction of the ascorbic acid in the processed juice and cannot meet the product requirement (Sinclair, 1984). Therefore, the synthesis of vitamin C needs to be added to the final product.

In the study, the ascorbic acid has been evaluated after the MTS treatment immediately and after 16 storage days at 4°C, which is presented in Figure 6.17 and Figure 6.18 respectively.

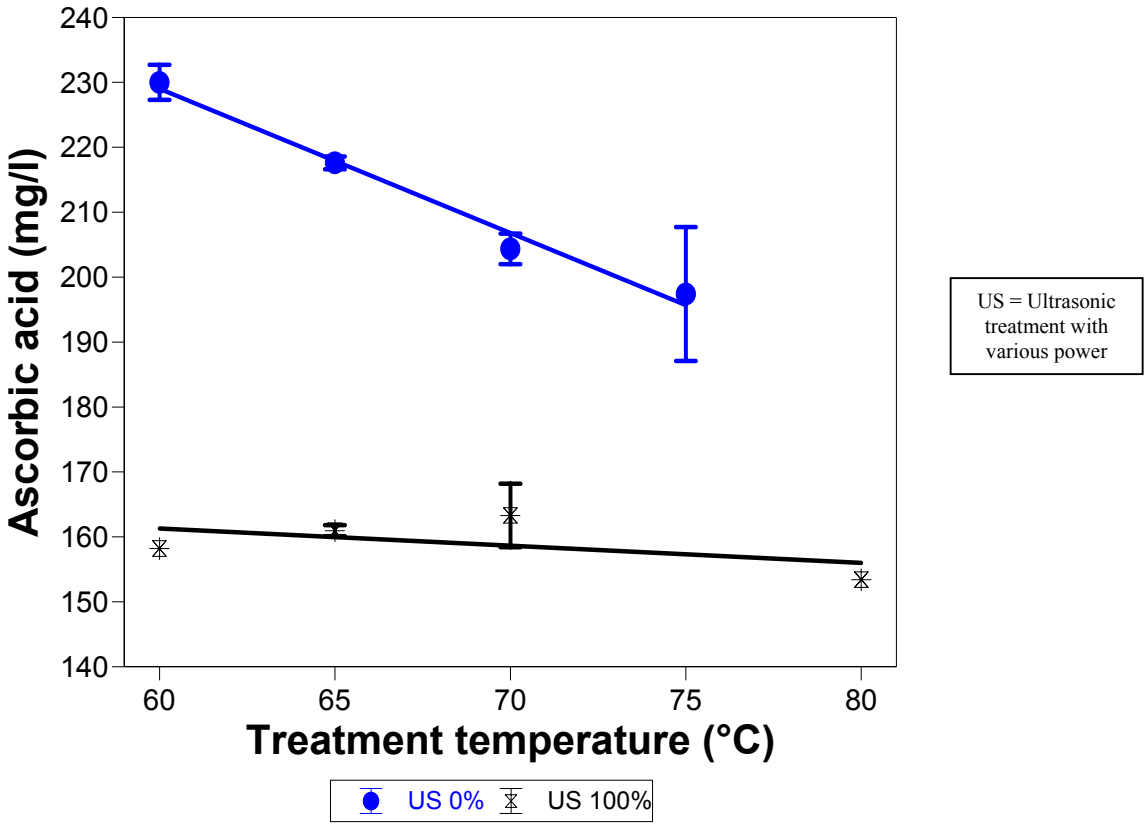


Figure 6.17: Ascorbic acid of the MTS treated juice at 300 kPa (immediate after treatment)

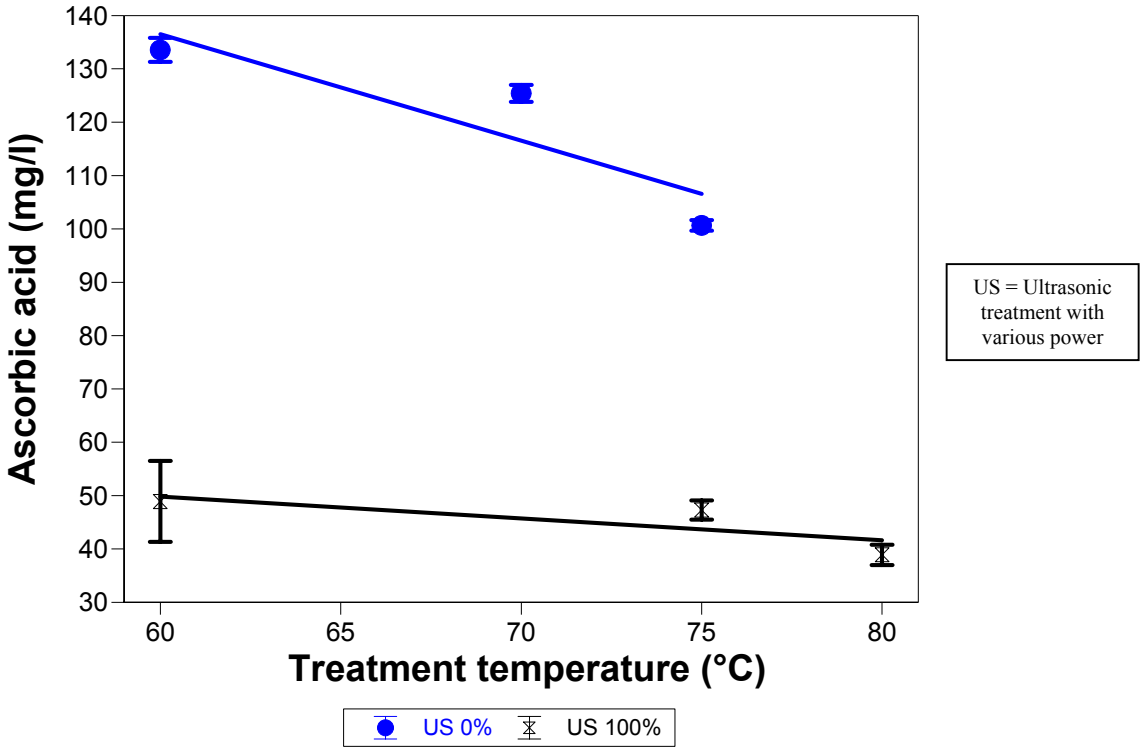


Figure 6.18: Ascorbic acid of the MTS treated juice at 300 kPa (after 16 storage days, 4°C)

The fresh lemon juice normally contains the ascorbic acid of ~400 mg/l (Sinclair, 1984; Ting and Rouseff, 1986). It has been found that the ascorbic acids were reduced after the MTS treatment and heat and pressure treatment. The greater ultrasonic power induced greater reduction at low temperature (60°C-70°C). It has been reported by Mason and Lorimer (1988) that the power ultrasound enhanced chemical reaction rate and yield. The oxidation reaction, which induced vitamin C loss during the process, was also accelerated by ultrasound (Mason and Lorimer, 1988).

The treated juice was stored at 4°C for 16 days and consequently analyzed the ascorbic acid. It has been found that the vitamin C was decreased during the storage. It was suggested that oxygen, the most destructive ingredient in the juice causing degradation of vitamin C, was presented (Sinclair, 1984). However, one of the enzyme found in citrus juice, dehydrogenase, can also cause vitamin C breakdown (Sinclair, 1984). It means that one need to concern more about the nutrition value of the MTS treated juice by the end of the process.

6.3.2 Effect of manothermosonication on pectinesterase of fresh strawberry juice

Figure 6.19 shows the percentage of the strawberry PE inactivation at various temperatures with ultrasound power 0%, 20%, 50% and 100%. The results showed the similar behaviours to those in fresh lemon PE process.

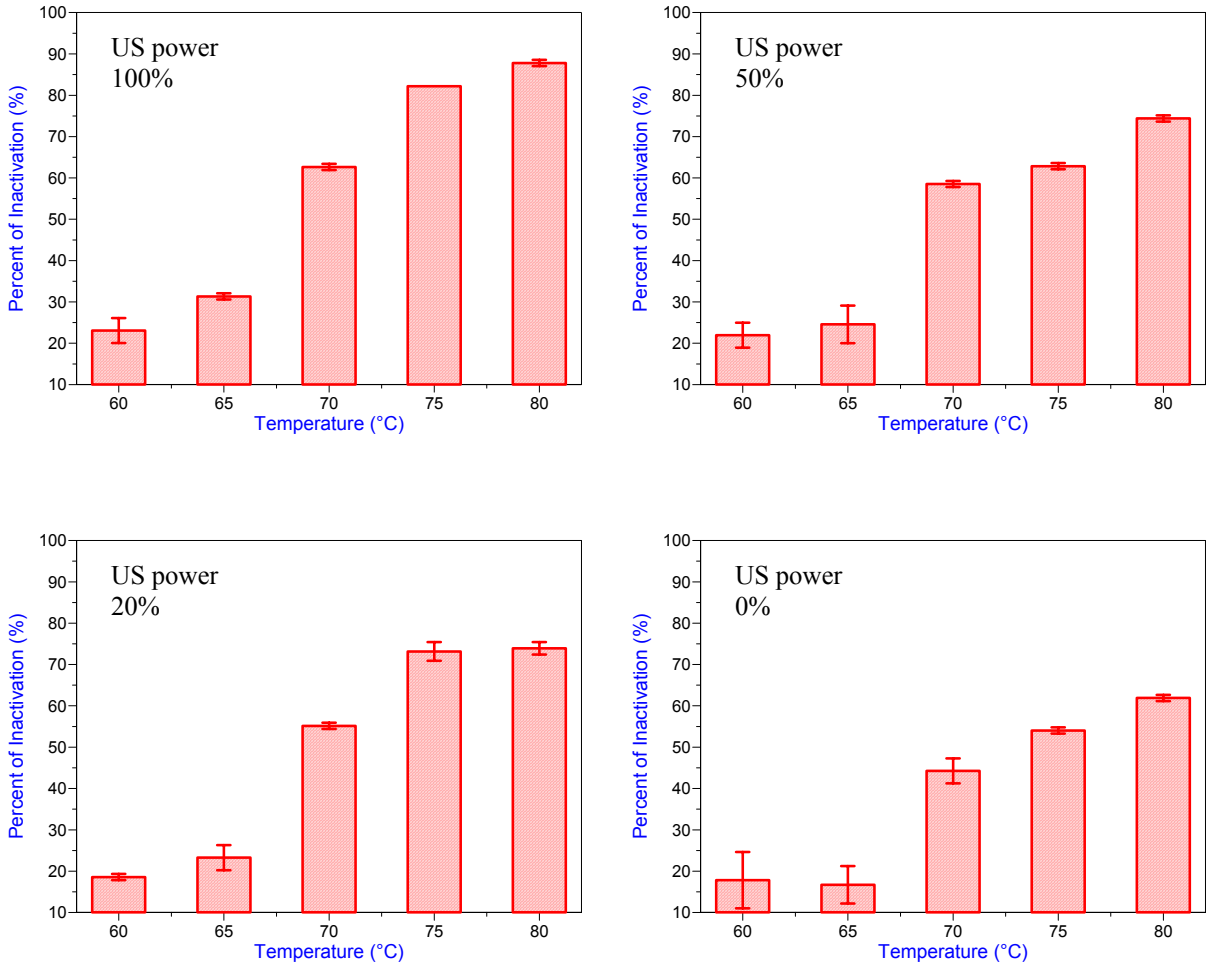


Figure 6.19: The percentage of strawberry PE inactivation by manothermosonicaion at 300 kPa

The MTS increasingly reduces enzyme activation. At 80°C, the enzyme was decreased by 73.9% with the ultrasonic power 20%, 74.4% with the power 50% and 87.8% with the power 100%. Therefore, the treatment condition, which mostly met the industrial requirement, was at the maximum power of ultrasound in combination with temperature 80°C and pressure 300 kPa.

Figure 6.20 presents the efficiency of ultrasonic power to the degree of fresh strawberry PE inactivation.

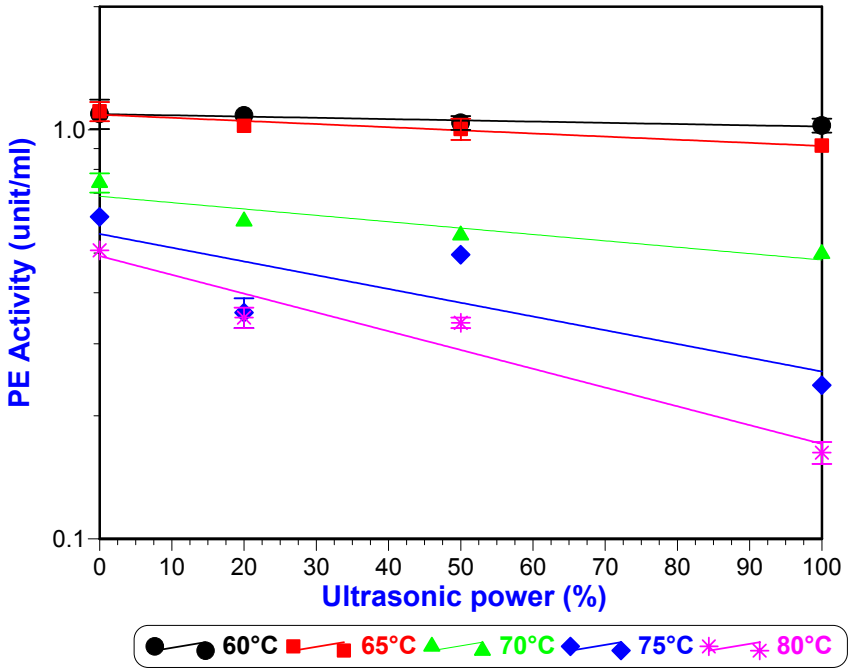


Figure 6.20: The effect of different level of ultrasound on the manothermosonication inactivation of thermoresistant strawberry PE

It has been shown that the inactivation of fresh strawberry was apparently increased with the increasing of ultrasonic power at all treatment temperatures. This was contrast to the fresh lemon PE inactivation at 60°C, where that ultrasound induced the activity of enzyme. However, increase in power of ultrasound at low temperature (~ 60-65°C) still has

less efficiency on the inactivation of enzyme. The increased amplitude could only enhance the inactivation process when higher temperature (> 70°C) was applied.

Some difficulties in the strawberry juice treatment could be defined as in the juice extraction and the treatment time adjustment. In the extraction process, it was necessary to be pre-treated by some pectic enzymes in order to allow the juice reaching the maximum yield. In this respect, more pectic enzymes were considered to be eliminated. This would also be the reason that higher MTS intensity was required in order to increase the enzyme inactivation. However, it was difficult to set up the slower flow rate (longer treatment time) at 300 kPa through this pilot plant.

6.3.3 Effect of manothermosonication on quality of strawberry juice

The absorbance (at 420 nm), colour, acidity, pH and conductivity of the strawberry juice were analyzed. It has been shown that no large MTS effect on the absorbance occurred after the treatment (Appendix A.14).

The measurement of the juice colour was investigated by the colorimeter (L-a-b values). It has been found no large difference of colour before and after the MTS treatment (Appendix A.15, Appendix A.16).

Moreover, the acidity of MTS treated and untreated juice was evaluated. It has been found that the acidity of the juice was not significantly changed after the MTS treatment (Appendix A.18). This is in accordance with the pH measurement, where the pH of the MTS treated juice showed no alteration upon the treatment (Appendix A.17).

The conductivity was also measured. There was no large difference of the conductivity between the untreated and treated juice (Appendix A.19).

6.3.4 Discussion

The heat, pressure and MTS treatment have been studied on the inactivation of fresh lemon and strawberry juice PE. It has been found that ultrasonic power has played an important role in the inactivation of both fresh-juice PE. The increased ultrasonic power effects in the increasing of PE inactivation at some certain level of temperatures (> 70°C). However, up

to 80°C and 300 kPa without ultrasound, the enzymes were rarely inactivated, which hardly met the industrial requirement. Therefore, ultrasound seems to be a tool to enhance the effectiveness of heat and pressure treatment.

Since heat treatment is known to be the primary implement that induces the protein unfolding and the enzyme inactivation, then the ultrasonic wave can improve this process by the cavitation. This cavitation makes the local hot spot, which leads to enzyme inactivation. The ultrasonic waves itself had less effect on that inactivation. However pressure enhances the bubble implosion intensity, which increase the inactivity kinetics. The inactivation of some enzymes by ultrasonic waves at ambient pressure and temperature has been previously reported by a number of researchers (Lopez et al., 1994; Vercet et al., 1997; Raso et al, 1998) and generally required long irradiation periods and the presence of oxygen.

Ultrasonic power has a great influence on the MTS inactivation of PE. The ultrasonic power 100% (~ 560 W) at 80°C and 300 kPa can make the PE destruction in lemon juice up to 91% and strawberry juice up to 87%. It is apparently that the inactivation of strawberry PE is more difficult than that in lemon juice.

The other properties of juice were not particularly changed after the MTS treatment, including pH, acidity, conductivity, absorbance, Brix, turbidity and colour. One more advantage is that the loss of cloud is clearly lower in the MTS treated juice than that in the untreated juice. The MTS processed juice also showed no viable micro-organism upon the storage, which meant that the MTS has, in parallel, accomplished the pasteurization.

Part V

Conclusion

Chapter 7

Conclusion

The inactivation of pectinesterase by ultrasonic wave is dependent on time of exposure, temperature, pressure, pH of medium, and amplitude of ultrasound. The inactivation is irreversible and the enzyme is not reactivated upon storage.

Several mechanisms can be operative in ultrasound inactivation of enzyme. Ultrasonic wave propagation through a liquid creates cavities (bubbles), mainly in weak spots created by dissolved gas molecules. The radii of these cavities expand and contract (cavitation) because of the induced pressure changes. Once a critical size has been reached, the cavity grows rapidly until it can no longer sustain itself and then collapses (cavitational collapse). The liquid stream produced in the vicinity of the bubble, either by bubble oscillation or implosion, often results in high temperature that can promote protein denaturation. In this aspect, increase in pressure can enhance the bubble implosion.

The inactivation effect of the mechanical damage produced by the cavitational collapse has been emphasized by Vercet et al., 1997. They proved that cavitational collapse is not always necessary for enzyme inactivation to occur as a consequence of critical hotspot (high temperature and high pressure) produced by ultrasonic waves. The polymers with high molecular mass can be degraded even in the absence of bubble collapse. Pressure and temperature have been taken into account to promote the chemical reactions involving free radicals that are formed by the decomposition of water inside the oscillating bubbles and lead to the inactivation of enzyme and micro-organism. Correspondingly, the ultrasound wave can also enhance this thermal and pressurize process since the ultrasound

itself can improve the fluid-to-particle convective heat transfer (Kuldiloke, 1995). Among these mechanisms, (pressure enhancing US, US enhancing heat and Heat & Pressure inducing chemical reaction), inactivation of the enzymes and destruction of microbial cells can be achieved.

Manothermosonication (MTS) is a method of combining temperature with moderate pressure and ultrasonics in the proper condition in order to inactivate enzyme and micro-organism. This combined method effects the enzyme inactivation synergistically. According to this study, MTS could inactivate lemon, tomato and strawberry PE at temperature where thermal inactivation was significant. At a given temperature, the MTS resulted in decrease of *D*-value for the inactivation, predominantly in lemon juice. Therefore, MTS is more effective than heat treatment in terms of shorter inactivation time of heat-resistant pectinesterase at the same operating temperature. The *z*-value of MTS treatment was comparatively higher than that of the heat treatment. This indicates that The MTS technique is more efficient at the lower treatment temperature. In addition, because of its higher efficiency in the inactivation of enzymes at temperature below 100°C, MTS can also be very useful to solve the quality problems caused by the heat-resistant pectinesterase. Moreover, MTS is able to impair the protection that several molecules offer to enzyme against heat inactivation. As result of this, MTS can inactivate enzymes that are protected by such molecules at temperatures ~ 70-80°C. However, sensitivity to MTS is suggested to be the result of a complex interaction of heat resistance PE and treatment medium.

This method could become an alternative to the conventional UHT treatments. The main advantage of MTS in lemon juice process is the satisfied quality of the juice. MTS treatment could maintain some fresh-liked properties of the juice, such as turbidity, colour, pH, Brix and cloud property. Nevertheless, the studies on food safety as well as the appropriate pre- and post-treatment should be investigated further.

Similar to the lemon juice process, the strawberry production required more precise data in terms of kinetic information. Consequently, the appropriate industrial equipment is necessary to be further designed in order to achieve more inactivation values.

In the tomato process, the MTS can inactivate PE, PG, POD and PPO in the combination with CO_2 gas. It is known that the CO_2 gas can reduce the pH of the treatment medium and consequently increase the enzyme inactivation. Various reports have

confirmed this increased PE inactivation rate under low pH values (Atkins and Rouse, 1953; Eagerman and Rouse, 1976). The MTS under CO_2 gas can be considered as a potential of MTS implement in the food process. Balaban et al. (1991) have demonstrated that the extent of the CO_2 inactivation depended on pressure, temperature and time. Besides, the CO_2 treatment could also notably reduce the reaction energy. However, more accurate inactivation mechanisms of the combined treatment of ultrasonic waves with CO_2 are necessary to be further carried out as well as the rheological properties and the MTS microbial destruction.

Part VI

Appendix

Appendix A

A.1: Heat inactivation of pectinesterase extracted from fresh lemon at various temperature (see figure 6.1)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0	11.90	0.00
	3	11.45	0.41
	18	10.85	0.06
	38	10.97	0.02
	48	10.57	0.19
	63	10.57	0.19
50	0	11.71	0.12
	3	11.24	0.04
	18	9.77	0.19
	33	9.46	0.05
	48	9.02	0.04
	63	8.11	0.09
60	0	11.15	0.51
	3	8.10	0.22
	18	2.45	0.06
	33	1.97	0.01
	48	1.95	0.00
	63	1.85	0.03
65	0	8.06	-
	3	7.37	-
	8	3.10	-
	18	2.00	-
	28	1.47	-
	38	1.55	-
70	0	11.53	0.05
	3	1.84	0.04
	8	1.55	0.03
	18	1.21	0.01
	28	1.12	0.01
	38	0.97	0.01
80	0	10.95	1.26
	3	0.47	0.06
	5	0.26	0.03
	18	0.24	0.02
	33	0.20	0.01
	63	0.16	0.01
90	0	10.84	0.06
	3	0.17	0.02
	5	0.13	0.01
	18	0.11	0.01
	33	0.09	0.00
	63	0.11	0.01

A.2: Inactivation of extracted pectinesterase from fresh lemon by thermosoniaction (ultrasound 20 kHz) (see figure 6.3)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0	10.10	0.01
	3	9.55	0.10
	6	8.84	0.29
	18	9.26	0.12
	33	8.66	0.11
50	0	13.32	0.31
	3	10.95	0.10
	18	7.38	0.40
	33	5.11	0.03
	48	3.56	0.08
	63	2.28	0.04
60	0	11.66	0.07
	3	9.97	0.25
	8	2.72	0.00
	18	1.82	0.02
	38	1.53	0.01
70	0	11.09	0.00
	3	3.74	0.13
	8	1.23	0.01
	18	0.97	0.02
	38	0.63	0.01
80	0	10.9	0.00
	3	1.25	0.01
	5	0.27	0.01
	18	0.09	0.01
	33	0.09	0.01
85	0	10.93	0.39
	3	0.35	0.10
	5	0.08	0.00
	8	0.09	0.00
	13	0.08	0.00
	18	0.067	0.00

A.3: Heat inactivation of pectinesterase in the continuous system (see figure 6.5)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.0	1.71	0.01
	1.0	1.70	0.00
	2.0	1.68	0.02
	5.0	1.70	0.00
50	0.0	1.71	0.02
	1.0	1.70	0.00
	2.0	1.70	0.00
	5.0	1.60	0.02
60	0.0	1.71	0.01
	1.0	1.70	0.00
	2.0	1.60	0.02
	5.0	1.60	0.02
70	0.0	1.71	0.01
	1.0	1.60	0.02
	2.0	1.54	0.02
	5.0	1.48	0.02
80	0.0	1.71	0.01
	1.0	1.16	0.02
	2.0	1.02	0.02
	5.0	0.60	0.02
90	0.0	1.71	0.01
	0.5	0.14	0.01
	1.0	0.02	0.01

A.4: Heat and pressure inactivation of pectinesterase (100 kPa) (see figure 6.6)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.0	1.92	0.02
	1.0	1.90	0.00
	2.0	1.87	0.03
	3.0	1.82	0.02
	5.0	1.80	0.00
50	0.0	1.92	0.02
	1.0	1.80	0.00
	2.0	1.67	0.03
	3.0	1.49	0.03
	5.0	1.40	0.00
60	0.0	1.97	0.03
	0.5	1.84	0.00
	1.0	1.72	0.02
	3.0	1.58	0.00
	5.0	1.09	0.03
65	0.0	1.94	0.00
	0.5	1.80	0.00
	1.0	1.64	0.00
	3.0	1.30	0.00
	5.0	0.91	0.03
70	0.0	1.90	0.00
	0.5	1.80	0.00
	1.0	1.61	0.03
	2.0	1.40	0.00
	3.0	1.09	0.03
	5.0	0.70	0.00
80	0.0	1.97	0.03
	0.5	1.80	0.00
	1.0	1.67	0.03
	2.0	1.40	0.00
	3.0	1.00	0.00
	5.0	0.58	0.00

A.5: Heat and pressure inactivation of pectinesterase (200 kPa) (see figure 6.6)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.0	1.90	0.00
	1.0	1.82	0.02
	2.0	1.72	0.02
	3.0	1.70	0.00
	5.0	1.70	0.00
50	0.0	1.90	0.00
	1.0	1.67	0.03
	2.0	1.61	0.03
	3.0	1.49	0.03
	5.0	1.30	0.00
60	0.0	1.90	0.00
	1.0	1.70	0.00
	2.0	1.52	0.00
	3.0	1.37	0.03
	5.0	1.00	0.00
70	0.0	1.90	0.00
	1.0	1.58	0.00
	2.0	1.43	0.03
	4.0	0.76	0.00
	5.0	0.58	0.00
75	0.0	1.90	0.00
	0.5	1.76	0.00
	1.0	1.58	0.00
	2.0	1.26	0.02
	5.0	0.50	0.02
80	0.0	1.90	0.00
	0.5	1.70	0.00
	1.0	1.61	0.03
	3.0	1.00	0.00
	5.0	0.43	0.03

A.6: Heat and pressure inactivation of pectinesterase (300 kPa) (see figure 6.6)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.00	1.70	0.00
	1.00	1.64	0.00
	2.00	1.61	0.03
	3.00	1.55	0.03
	5.00	1.49	0.03
50	0.00	1.70	0.00
	1.00	1.58	0.00
	2.00	1.40	0.00
	3.00	1.27	0.10
	5.00	1.09	0.03
60	0.00	1.70	0.00
	0.25	1.64	0.00
	0.50	1.58	0.00
	1.00	1.49	0.03
	2.00	1.28	0.00
65	0.00	1.84	0.00
	0.25	1.70	0.00
	0.50	1.67	0.03
	1.00	1.40	0.00
	2.00	1.31	0.03
	5.00	0.64	0.00
70	0.00	1.84	0.00
	0.25	1.70	0.00
	0.50	1.67	0.03
	1.00	1.49	0.03
	2.00	1.20	0.02
80	0.00	1.70	0.00
	0.25	1.46	0.00
	0.50	1.43	0.03
	1.00	1.40	0.00
	2.00	1.12	0.00

A.7: Manothermosonication inactivation of lemon pectinesterase (100 kPa, ultrasound 100% amplitude) (see figure 6.9)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.0	1.92	0.02
	1.0	1.77	0.03
	2.0	1.61	0.03
	3.0	1.49	0.03
	5.0	1.30	0.00
50	0.0	1.92	0.02
	1.0	1.70	0.00
	2.0	1.55	0.03
	3.0	1.40	0.00
	5.0	1.09	0.03
60	0.0	1.97	0.03
	0.5	1.34	0.00
	1.0	1.24	0.00
	3.0	0.58	0.00
	5.0	0.49	0.00
65	0.0	1.94	0.00
	0.5	1.30	0.00
	1.0	0.94	0.00
	3.0	0.38	0.02
	5.0	0.24	0.00
70	0.0	1.90	0.00
	0.5	1.00	0.00
	1.0	0.40	0.00
	2.0	0.18	0.00
	3.0	0.10	0.00
80	0.0	1.97	0.03
	0.5	0.15	0.02
	1.0	0.13	0.00
	2.0	0.01	0.01

A.8: Manothermosonication inactivation of lemon pectinesterase (200 kPa, ultrasound 100% amplitude) (see figure 6.9)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.0	1.90	0.00
	1.0	1.70	0.10
	2.0	1.50	0.02
	3.0	1.30	0.00
	5.0	1.00	0.00
50	0.0	1.90	0.00
	1.0	1.60	0.00
	2.0	1.40	0.00
	3.0	1.29	0.05
	5.0	0.80	0.00
60	0.0	1.92	0.02
	1.0	1.10	0.00
	2.0	0.30	0.00
	3.0	0.25	0.00
	5.0	0.11	0.01
65	0.0	1.92	0.02
	1.0	0.30	0.00
	2.0	0.18	0.00
	4.0	0.10	0.00
	5.0	0.07	0.01
70	0.0	1.90	0.00
	0.5	0.41	0.01
	1.0	0.28	0.00
	2.0	0.12	0.01
	5.0	0.09	0.00
80	0.0	1.90	0.00
	0.5	0.24	0.00
	1.0	0.10	0.00
	3.0	0.09	0.00
	5.0	0.07	0.00

A.9: Manothermosonication inactivation of lemon pectinesterase (300 kPa, ultrasound 100% amplitude) (see figure 6.9)

Temperature (°C)	Time (min)	Activity (unit/ml)	Standard error
40	0.00	1.70	0.00
	1.00	1.30	0.00
	2.00	1.20	0.02
	3.00	1.03	0.00
	5.00	1.00	0.00
50	0.00	1.70	0.00
	1.00	1.20	0.02
	2.00	1.10	0.00
	3.00	1.00	0.00
	5.00	0.60	0.02
60	0.00	1.70	0.00
	0.25	0.88	0.00
	0.50	0.70	0.00
	1.00	0.60	0.00
	2.00	0.22	0.12
65	0.00	1.84	0.02
	0.25	0.70	0.00
	0.50	0.60	0.00
	1.00	0.30	0.00
	2.00	0.12	0.00
70	0.00	1.84	0.00
	0.25	0.50	0.05
	0.50	0.21	0.00
	1.00	0.13	0.00
	2.00	0.10	0.00
80	0.00	1.70	0.00
	0.25	0.30	0.00
	0.50	0.12	0.00
	1.00	0.10	0.00
	2.00	0.07	0.01

A.10: Activity and percent of inactivation of PE in fresh lemon juice after manothermosonication treatment (see figure 6.16)

Ultrasonic amplitude (%)	Sample no.	Actual power (W)	Temperature at the outlet (°C)	Pressure (kPa)	Flowrate (L/h)	Enzyme activity (Unit/ml)	Standard diviation	Percent of inactivation (%)
0	19	0	60	300	10.2	1.09	0.04	53.38
	18	0	65	300	10.2	1.86	0.01	20.78
	17	0	70	300	10.2	1.01	0.06	57.21
	16	0	75	300	10.2	0.93	0.03	60.18
20	11	252	60	300	10.2	1.50	0.01	36.25
	12	238	65	300	10.2	1.14	0.02	51.39
	13	254	70	300	10.2	1.02	0.09	56.67
	14	235	75	300	10.2	0.53	0.01	77.59
	15	238	80	300	10.2	0.38	0.01	83.54
50	10	330	60	300	10.2	1.38	0.23	41.49
	9	330	65	300	10.2	1.37	0.01	41.81
	8	324	70	300	10.2	0.45	0.01	80.67
	7	297	75	300	10.2	0.44	0.03	81.10
	6	297	80	300	10.2	0.31	0.00	87.04
100	1	565	60	300-320	38.0	1.68	0.01	28.43
	2	535	65	300	10.0	1.08	0.05	53.94
	3	535	70	300	10.2	0.49	0.00	78.99
	4	515	75.5	300	10.2	0.45	0.01	80.67
	5	521	80.8-81	300	10.2	0.21	0.02	91.18

Note: Initial activity = 2.34 unit/ml. The numbers of the samples were represented as the order that the samples have been taken (see temperature profile in appendix B.4)

A.11: The chemical analysis of fresh squeezed lemon juice (3 to 5 storage days at 4°C)

Method of treatment	Actual power (Watt)	Flowrate (L/h)	Turbidity (TE/F) before centrifugation	Turbidity (TE/F) after centrifugation	Brix	Density (g/cm3)	pH	Colour L value	a value	b value
60°C, 100% Ultrasound	565	38	50x64.0	50x57.0	8.0	1.0390	2.15	42.22	-0.77	11.39
3 days storage								42.16	-0.66	11.30
65°C, 100% Ultrasound	535	10	50x64.0	50x57.0	7.7	1.0365	2.11	43.52	-0.48	12.43
3 days storage								43.42	-1.45	12.34
70°C, 100% Ultrasound	535	10.2	50x65.0	50x60.0	8.1	1.0383	2.10	44.15	-0.37	13.18
3 days storage								44.22	-0.42	13.30
75°C, 100% Ultrasound	515	10.2	50x63.0	50x55.0	8.2	1.0395	2.10	44.25	-0.35	13.43
3 days storage								44.12	-0.36	13.46
80°C, 100% Ultrasound	521	10.2	50x62.5	50x54.5	8.2	1.0395	2.02	44.85	-0.24	14.39
3 days storage								44.89	-0.20	14.35
80°C, 50% Ultrasound	297	10.2	50x64.5	50x60.0	8.1	1.0398	2.05	43.06	-0.52	12.31
3 days storage								43.16	-0.53	12.43
75°C, 50% Ultrasound	297	10.2	50x64.0	50x59.5	8.1	1.0389	2.02	43.26	-0.57	12.51
3 days storage								43.24	-0.46	12.47
70°C, 50% Ultraosund	324	10.2	50x67.0	50x60.0	8.1	1.0389	2.10	42.58	-0.52	11.58
5 days storage								42.69	-0.54	11.22
65°C, 50% Ultraosund	330	10.2	50x64.5	50x56.0	8.2	1.0388	2.10	42.76	-0.56	11.66
5 days storage								42.77	-0.54	11.68
60°C, 50% Ultraosund	330	10.2	50x63.0	50x55.5	8.1	1.0388	2.10	42.64	-0.56	11.52
5 days storage								42.70	-0.55	11.55
60°C, 20% Ultraosund	252	10.2	50x65.0	50x58.5	8.1	1.0388	2.12	42.52	-0.50	12.04
5 days storage								42.52	-0.51	12.10
65°C, 20% Ultraosund	238	10.2	50x64.5	50x58.5	8.2	1.0389	2.10	42.05	-0.64	11.40
5 days storage			50x65.0	50x57.5				41.97	-0.69	11.43
70°C, 20% Ultraosund	254	10.2	50x67.0	50x62.0	8.1-8.2	1.0390	2.10	42.09	-0.72	11.22
5 days storage				50x58.6				42.07	-0.71	11.22
75°C, 20% Ultraosund	235	10.2	50x67.5	50x63.0	8.1	1.0388	2.12	42.08	-0.74	11.08
5 days storage				50x62.0				42.06	-0.66	11.01
80°C, 20% Ultraosund	238	10.2	50x63.5	50x56.0	8.2	1.0395	2.10	42.06	-0.71	11.10
5 days storage								42.06	-0.74	11.12
75°C, 0% Ultraosund	0	10.2	50x62.5	50x53.0	8.1	1.0389	2.10	42.62	-0.81	11.23
5 days storage								42.65	-0.78	11.27
70°C, 0% Ultraosund	0	10.2	50x61.5	50x54.0	8.1	1.0389	2.11	40.63	-0.75	11.03
5 days storage			50x60.0					40.61	-0.85	11.10
65°C, 0% Ultraosund	0	10.2	50x62.5	50x55.0	8.1	1.0389	2.11	40.54	-0.88	10.96
5 days storage				50x56.0				40.57	-0.89	10.98
60°C, 0% Ultraosund	0	10.2	50x66.0	50x53.0	8.1	1.0390	2.12	40.66	-1.15	10.23
5 days storage								40.63	-1.09	10.22
Untreated juice	0	-	50x64.0	50x51.0	8.1	1.0395	2.20	41.46	-1.05	10.84
3 days storage								41.31	-1.10	10.67

A.12: The chemical analysis of fresh squeezed lemon juice (16 storage days at 4°C)

Method of treatment	Actual power (Watt)	Flowrate (L/h)	Turbidity (TE/F) before centrifugation	Turbidity (TE/F) after centrifugation	Brix	Density (g/cm3)	pH	Clarification (420 nm)	Colour			Colour after centrifugation		
									L value	a value	b value	L value	a value	b value
60°C	565	38.0	50x62.5	50x49.5	8.0	1.0376	2.30	0.817	41.40	-0.33	10.63	43.57	-0.11	-12.87
100% Ultrasound									41.38	-0.27	10.59	43.68	-0.16	13.13
65°C	535	10.0	50x62.5	50x52.0	7.7	1.0370	2.30	0.958	43.09	-0.09	11.59	45.20	-0.03	14.04
100% Ultrasound									43.04	0.07	-11.62	45.56	0.00	14.38
70°C	535	10.2	50x69.0	50x58.0	8.1	1.0388	2.30	0.899	43.56	-0.11	12.05	45.80	0.07	14.84
100% Ultrasound									43.74	-0.24	12.26	45.99	0.11	15.09
75°C	515	10.2	50x66.0	50x56.0	8.2	1.0392	2.30	0.931	44.01	0.01	12.77	46.09	0.10	15.46
100% Ultrasound									44.03	0.01	12.88	46.27	0.07	15.64
80°C	521	10.2	50x67.0	50x54.0	8.2	1.0395	2.30	0.891	44.60	0.07	13.61	46.59	0.16	16.32
100% Ultrasound									44.73	-0.04	13.84	46.73	0.12	16.58
75°C	0	10.2	50x62.5	50x51.5	8.1	1.0392	2.32	0.745	44.34	-0.72	11.74	42.72	-0.33	12.77
0% Ultraosund									41.43	-0.69	11.9	42.92	-0.31	13.05
70°C	0	10.2	50x67.5	50x47.0	8.1	1.0392	2.35	0.644	40.72	-0.95	10.43	42.93	-0.33	12.80
0% Ultraosund									40.54	-0.96	10.38	42.93	-0.28	12.82
65°C	0	10.2	50x64.0	50x45.0	8.1	1.0391	2.35	0.643	40.72	-0.82	10.15	42.86	-0.21	12.59
0% Ultraosund									40.71	-0.90	10.32	43.35	-0.25	12.87
60°C	0	10.2	50x63.0	50x48.0	8.1	1.0391	2.40	0.700	40.78	-0.95	10.67	43.62	-0.34	11.93
0% Ultraosund									40.76	-0.68	10.74	42.75	-0.31	12.17
Untreated juice	0	-	50x65.0	50x48.0	8.3	1.0390	2.30	0.419	41.42	-0.82	10.71	46.05	-0.10	14.18
									41.48	-0.81	10.61	46.25	-0.08	14.36

A.13: Activity and percent of inactivation of PE in fresh strawberry juice after manothermosonication treatment (see figure 6.18)

Ultrasound amplitude (%)	Actual power (W)	Temperature at the outlet (°C)	pressure (kPa)	Enzyme activity (Unit/ml)	Standard diviation	Percent of inactivation (%)
0	0	60	270	1.09	0.09	17.82
	0	65	270	1.11	0.06	16.69
	0	70	290	0.74	0.04	44.27
	0	75	260	0.61	0.01	54.03
	0	80	240	0.51	0.01	61.91
20	241.5	60	290	1.08	0.01	18.57
	242.8	65	280	1.02	0.04	23.26
	238.6	70	290	0.59	0.01	55.16
	241.5	75	290	0.35	0.03	73.17
	243.8	80	300	0.34	0.02	73.92
50	366.9	60	270	1.04	0.04	21.95
	361.3	65	280	1.01	0.06	24.57
	369.8	70	280	0.55	0.01	58.53
	311.5	75	280	0.49	0.01	62.85
	369.1	80	280	0.33	0.01	74.39
100	663.0	60	320	1.02	0.04	23.07
	666.0	65	310	0.91	0.01	31.33
	648.0	70	300	0.49	0.01	62.66
	668.0	75	300	0.23	0.00	82.17
	583.0	80	260	0.16	0.01	87.80

Note: The flowrate was approximately 10 L/h. However, it was changing in order to keep the constant pressure.

A.14: The absorbance of strawberry juice before and after heat treatment and MTS treatment

Sample	before treatment				after treatment			
	420 nm mean value	500 nm mean value	540 nm mean value	700 nm mean value	420 nm mean value	500 nm mean value	540 nm mean value	700 nm mean value
Fresh juice	0.932 0.976	1.440 1.485	0.728 0.764	0.035 0.055	0.954 0.805	1.463 0.070	0.746 0.971	0.045 0.832
Pectinised juice	1.105 1.167	1.567 1.622	0.846 0.892	0.085 0.114	1.136 0.852	1.595 0.098	0.869 0.927	0.099 4.055
45°C without pressure	1.041 0.964	1.454 1.477	0.812 0.753	0.080 0.043	1.003 0.940	8.013 1.449	0.782 0.738	0.061 0.034
60°C without pressure	0.916 0.929	1.422 1.438	0.723 0.731	0.025 0.027	0.923 0.958	1.430 1.454	0.727 0.749	0.026 0.042
65°C without pressure	0.987 1.039	1.470 1.551	0.768 0.813	0.057 0.060	1.013 1.019	1.510 1.506	0.790 0.794	0.058 0.063
70°C without pressure	1.000 1.019	1.461 1.484	0.775 0.788	0.067 0.070	1.009 0.981	1.473 1.432	0.782 0.755	0.068 0.058
75°C without pressure	0.942 1.001	1.380 1.454	0.723 0.771	0.047 0.063	0.972 1.009	1.417 1.447	0.747 0.773	0.055 0.069
80°C without pressure	1.016 1.035	1.440 1.464	0.774 0.788	0.076 0.080	0.781 0.810	0.078 1.141	0.795 0.608	0.609 0.063
60°C, US 0% 300 kPa	0.585 0.629	0.818 0.880	0.427 0.460	0.046 0.049	0.607 0.619	0.849 0.889	0.444 0.457	0.047 0.036
65°C, US 0% 300 kPa	0.609 0.645	0.899 0.954	0.453 0.482	0.023 0.026	0.627 0.861	0.927 1.286	0.468 0.635	0.024 0.036
70°C, US 0% 300 kPa	1.076 1.108	1.619 1.668	0.787 0.811	0.047 0.049	1.092 0.948	1.644 1.417	0.799 0.707	0.048 0.039
75°C, US 0% 300 kPa	0.788 0.808	1.166 1.195	0.602 0.617	0.029 0.030	0.798 0.882	1.181 1.269	0.609 0.668	0.029 0.048
80°C, US 0% 300 kPa	0.955 0.996	1.342 1.369	0.718 0.747	0.066 0.090	0.975 1.041	1.355 1.475	0.733 0.791	0.078 0.082
60°C, US 20% 300 kPa	1.086 1.159	1.582 1.682	0.834 0.895	0.073 0.083	0.864 0.892	0.078 0.109	0.878 1.046	0.094 0.861
65°C, US 20% 300 kPa	1.185 1.214	1.596 1.630	0.889 0.911	0.136 0.146	1.199 0.831	1.613 0.100	0.900 0.908	0.141 0.775
70°C, US 20% 300 kPa	0.971 1.000	1.433 1.469	0.750 0.770	0.054 0.058	0.986 0.998	1.451 1.448	0.760 0.763	0.056 0.062
75°C, US 20% 300 kPa	0.995 1.015	1.428 1.455	0.755 0.771	0.065 0.069	1.005 0.994	1.442 1.425	0.763 0.758	0.067 0.067
80°C, US 20% 300 kPa	0.973 0.991	1.394 1.419	0.745 0.758	0.065 0.069	0.982 0.971	1.406 1.433	0.752 0.752	0.067 0.059
60°C, US 50% 300 kPa	0.950 0.991	1.446 1.482	0.745 0.771	0.049 0.059	0.971 1.028	1.464 1.495	0.758 0.790	0.054 0.073
65°C, US 50% 300 kPa	1.065 1.091	1.508 1.548	0.809 0.845	0.087 0.089	1.078 1.014	1.528 1.468	0.827 0.782	0.088 0.066
70°C, US 50% 300 kPa	0.936 0.968	1.388 1.438	0.719 0.746	0.044 0.047	0.952 1.074	1.413 1.523	0.733 0.817	0.045 0.085
75°C, US 50% 300 kPa	1.180 1.185	1.608 1.609	0.888 0.890	0.123 0.125	1.183 1.081	1.608 1.503	0.889 0.815	0.124 0.095
80°C, US 50% 300 kPa	0.977 1.015	1.396 1.453	0.741 0.774	0.065 0.068	0.996 1.029	1.424 1.464	0.758 0.782	0.066 0.075
60°C, US 100% 300 kPa	1.044 1.067	1.476 1.507	0.789 0.807	0.081 0.085	1.055 0.991	1.491 1.442	0.798 0.756	0.083 0.058
65°C, US 100% 300 kPa	0.915 0.932	1.377 1.402	0.705 0.718	0.031 0.032	0.924 1.019	1.389 1.452	0.712 0.770	0.032 0.067
70°C, US 100% 300 kPa	1.107 1.124	1.503 1.527	0.822 0.835	0.102 0.103	1.115 1.153	1.515 1.535	0.828 0.849	0.102 0.114
75°C, US 100% 300 kPa	1.182 1.194	1.542 1.557	0.863 0.872	0.124 0.126	1.188 1.056	1.549 1.441	0.868 0.783	0.125 0.084
80°C, US 100% 300 kPa	0.919 0.943	1.324 1.356	0.693 0.710	0.042 0.043	0.931 0.710	1.340 0.043	0.702 0.710	0.043 0.043

A.15: Colour measurement of strawberry juice after heat and manothermosonication treatment

Turbid juice

method of treatment	Colour		
	L-value	a-value	b-value
Pectinised juice	27.89 ± 0.06	14.44 ± 0.12	9.57 ± 0.06
45°C without pressure	27.89 ± 0.03	15.01 ± 0.08	9.63 ± 0.10
60°C without pressure	27.67 ± 0.03	14.68 ± 0.08	9.90 ± 0.03
65°C without pressure	27.46 ± 0.01	14.35 ± 0.07	9.79 ± 0.04
70°C without pressure	27.75 ± 0.02	14.57 ± 0.03	9.86 ± 0.07
75°C without pressure	27.98 ± 0.06	14.27 ± 0.06	9.26 ± 0.09
80°C without pressure	26.03 ± 0.04	11.90 ± 0.04	7.43 ± 0.05
60°C, US 0% 300 kPa	27.37 ± 0.03	11.02 ± 0.10	7.44 ± 0.10
65°C, US 0% 300 kPa	27.48 ± 0.04	14.04 ± 0.04	9.20 ± 0.12
70°C, US 0% 300 kPa	27.41 ± 0.04	11.73 ± 0.05	7.91 ± 0.03
75°C, US 0% 300 kPa	25.90 ± 0.05	11.58 ± 0.03	7.58 ± 0.02
80°C, US 0% 300 kPa	27.74 ± 0.04	14.94 ± 0.07	9.57 ± 0.07
60°C, US 20% 300 kPa	27.71 ± 0.07	15.21 ± 0.09	9.87 ± 0.06
65°C, US 20% 300 kPa	25.90 ± 0.05	11.58 ± 0.03	7.58 ± 0.02
70°C, US 20% 300 kPa	27.69 ± 0.03	14.46 ± 0.12	9.79 ± 0.05
75°C, US 20% 300 kPa	27.69 ± 0.01	14.28 ± 0.05	9.51 ± 0.03
80°C, US 20% 300 kPa	27.12 ± 0.08	13.53 ± 0.08	8.83 ± 0.08
60°C, US 50% 300 kPa	27.60 ± 0.02	14.21 ± 0.03	8.94 ± 0.04
65°C, US 50% 300 kPa	27.30 ± 0.01	13.92 ± 0.09	9.30 ± 0.03
70°C, US 50% 300 kPa	27.89 ± 0.06	14.44 ± 0.12	9.57 ± 0.06
75°C, US 50% 300 kPa	27.18 ± 0.03	13.64 ± 0.07	8.99 ± 0.09
80°C, US 50% 300 kPa	27.52 ± 0.03	13.79 ± 0.10	8.99 ± 0.06
60°C, US 100% 300 kPa	27.03 ± 0.03	13.83 ± 0.01	9.37 ± 0.05
65°C, US 100% 300 kPa	-	-	-
70°C, US 100% 300 kPa	27.81 ± 0.17	13.65 ± 0.02	8.85 ± 0.25
75°C, US 100% 300 kPa	27.19 ± 0.03	13.78 ± 0.03	8.99 ± 0.06
80°C, US 100% 300 kPa	27.14 ± 0.03	13.06 ± 0.06	8.51 ± 0.03

A.16: Colour measurement of strawberry juice after heat and manothermosonication treatment

Clear juice

method of treatment	Colour		
	L-value	**a-value**	**b-value**
60°C without pressure	30.94 ± 0.38	32.04 ± 0.79	20.24 ± 0.85
65°C without pressure	29.64 ± 0.93	29.79 ± 2.14	18.22 ± 1.79
70°C without pressure	29.71 ± 0.48	30.10 ± 0.93	18.61 ± 0.97
75°C without pressure	30.14 ± 0.47	31.01 ± 0.52	19.42 ± 0.43
80°C without pressure	-	-	-
60°C, US 0% 300 kPa	34.32 ± 1.01	32.49 ± 1.37	26.65 ± 1.89
65°C, US 0% 300 kPa	34.26 ± 0.77	33.98 ± 1.08	26.57 ± 1.43
70°C, US 0% 300 kPa	-	-	-
75°C, US 0% 300 kPa	31.36 ± 0.68	32.33 ± 1.30	21.47 ± 1.38
80°C, US 0% 300 kPa	-	-	-
60°C, US 20% 300 kPa	-	-	-
65°C, US 20% 300 kPa	-	-	-
70°C, US 20% 300 kPa	29.95 ± 0.31	30.71 ± 0.62	19.08 ± 0.66
75°C, US 20% 300 kPa	30.11 ± 0.55	30.29 ± 0.73	18.83 ± 0.61
80°C, US 20% 300 kPa	29.68 ± 0.84	29.69 ± 1.79	18.63 ± 1.61
60°C, US 50% 300 kPa	30.76 ± 0.56	31.87 ± 1.23	20.27 ± 1.16
65°C, US 50% 300 kPa	28.96 ± 0.34	28.56 ± 0.84	17.37 ± 0.63
70°C, US 50% 300 kPa	30.20 ± 0.56	31.19 ± 0.82	19.63 ± 0.70
75°C, US 50% 300 kPa	28.77 ± 0.66	27.37 ± 0.83	16.2 0± 0.93
80°C, US 50% 300 kPa	29.52 ± 0.78	29.49 ± 1.58	18.38 ± 1.39
60°C, US 100% 300 kPa	29.07 ± 0.17	28.61 ± 0.50	17.42 ± 0.25
65°C, US 100% 300 kPa	30.89 ± 0.38	32.20 ± 0.96	20.76 ± 0.94
70°C, US 100% 300 kPa	28.40 ± 0.26	27.18 ± 0.44	16.31 ± 0.31
75°C, US 100% 300 kPa	-	-	-
80°C, US 100% 300 kPa	30.10 ± 0.57	29.26 ± 2.03	17.93 ± 2.13

A.17: pH of strawberry juice before and after heat treatment and manothermosonication treatment (300 kPa)

Sample	1st pH	2nd pH	3rd pH	4th pH	mean pH	Standard deviation
Fresh juice	3.20	3.20	3.19	3.19	3.19	0.01
Pectinised juice	3.21	3.21	3.22	3.20	3.21	0.01
45°C, no pressure	3.22	3.20	3.19	3.19	3.20	0.01
60°C, no pressure	3.20	3.22	3.20	3.22	3.21	0.01
65°C, no pressure	3.25	3.21	3.21	3.21	3.22	0.02
70°C, no pressure	3.22	3.21	3.22	3.21	3.21	0.01
75°C, no pressure	3.22	3.21	3.21	3.21	3.21	0.01
80°C, no pressure	3.22	3.21	3.19	3.21	3.21	0.01
60°C, US 0%	3.31	3.32	3.30	3.30	3.31	0.01
65°C, US 0%	3.31	3.31	3.29	3.30	3.30	0.01
70°C, US 0%	3.25	3.27	3.28	3.28	3.27	0.01
75°C, US 0%	3.30	3.28	3.29	3.26	3.28	0.01
80°C, US 0%	3.22	3.25	3.22	3.22	3.23	0.01
60°C, US 20%	3.30	3.25	3.22	3.20	3.24	0.04
65°C, US 20%	3.25	3.22	3.22	3.20	3.22	0.02
70°C, US 20%	3.16	3.22	3.22	3.22	3.20	0.03
75°C, US 20%	3.25	3.22	3.20	3.21	3.22	0.02
80°C, US 20%	3.22	3.21	3.21	3.21	3.21	0.01
60°C, US 50%	3.20	3.21	3.19	3.20	3.20	0.01
65°C, US 50%	3.20	3.21	3.19	3.20	3.20	0.01
70°C, US 50%	3.32	3.28	3.25	3.25	3.27	0.03
75°C, US 50%	3.15	3.20	3.22	3.21	3.19	0.03
80°C, US 50%	3.25	3.24	3.20	3.21	3.22	0.02
60°C, US 100%	3.19	3.21	3.21	3.21	3.20	0.01
65°C, US 100%	3.30	3.25	3.25	3.24	3.26	0.02
70°C, US 100%	3.21	3.19	3.20	3.19	3.19	0.01
75°C, US 100%	3.22	3.22	3.21	3.22	3.21	0.01
80°C, US 100%	3.24	3.22	3.22	3.23	3.22	0.01

A.18: The PE acidity of strawberry juice before and after heat treatment and manothermosonication treatment (300 kPa)

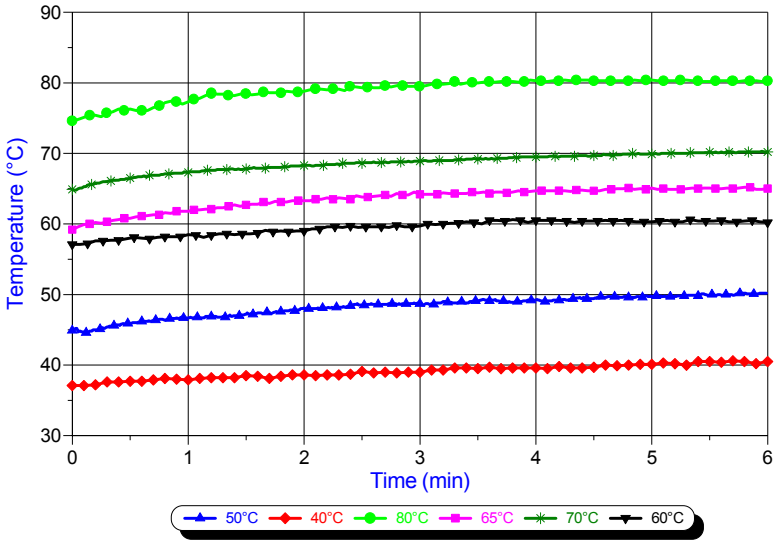
Sample	1st Acidity	2nd Acidity	mean value	Standard deviation
Fresh juice	18.10	18.10	18.10	0.00
Pectinised juice	18.15	18.05	18.10	0.07
45°C, no pressure	17.90	-	17.90	-
60°C, no pressure	18.30	18.40	18.35	0.07
65°C, no pressure	17.65	17.75	17.70	0.07
70°C, no pressure	17.60	17.80	17.70	0.14
75°C, no pressure	16.70	17.70	17.20	0.71
80°C, no pressure	17.70	17.80	17.75	0.07
60°C, US 0%	10.80	11.00	10.90	0.14
65°C, US 0%	12.50	12.50	12.50	0.00
70°C, US 0%	14.80	15.00	14.90	0.14
75°C, US 0%	16.40	16.45	16.43	0.04
80°C, US 0%	17.08	17.20	17.14	0.08
60°C, US 20%	18.20	18.30	18.25	0.07
65°C, US 20%	17.80	18.10	17.95	0.21
70°C, US 20%	18.10	18.20	18.15	0.07
75°C, US 20%	17.70	17.80	17.75	0.07
80°C, US 20%	17.55	17.70	17.63	0.11
60°C, US 50%	17.70	17.85	17.78	0.11
65°C, US 50%	17.50	17.70	17.60	0.14
70°C, US 50%	17.30	17.60	17.45	0.21
75°C, US 50%	17.50	17.80	17.65	0.21
80°C, US 50%	18.10	18.20	18.15	0.07
60°C, US 100%	17.30	17.70	17.50	0.28
65°C, US 100%	17.50	17.70	17.60	0.14
70°C, US 100%	17.90	17.60	17.75	0.21
75°C, US 100%	17.90	18.10	18.00	0.14
80°C, US 100%	17.70	17.90	17.80	0.14

A.19: The conductivity of strawberry juice before and after heat treatment and manothermosonication treatment (300

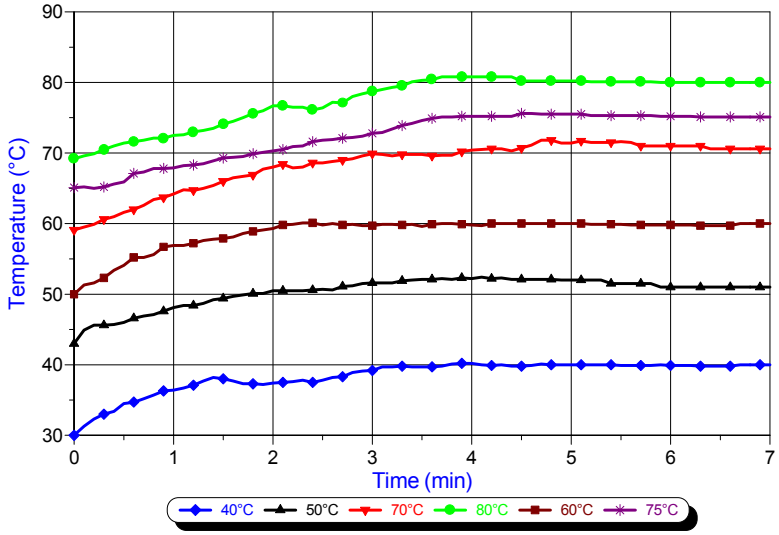
Sample	1st Conductivity (mS/cm)	2nd Conductivity (mS/cm)	3rd Conductivity (mS/cm)	4th Conductivity (mS/cm)	mean value (mS/cm)	standard deviation
Fresh juice	4.24	4.24	4.23	4.23	4.23	0.01
Pectinised juice	4.18	4.18	4.18	4.17	4.17	0.01
45°C, no pressure	4.27	4.26	4.26	4.26	4.26	0.01
60°C, no pressure	4.11	4.11	4.11	4.11	4.11	0.00
65°C, no pressure	4.06	4.07	4.07	4.07	4.06	0.01
70°C, no pressure	4.07	4.07	4.07	4.07	4.07	0.00
75°C, no pressure	4.09	4.09	4.09	4.09	4.09	0.00
80°C, no pressure	4.19	4.19	4.20	4.20	4.19	0.01
60°C, US 0%	3.23	3.23	3.23	3.23	3.23	0.00
65°C, US 0%	3.49	3.48	3.49	3.49	3.48	0.01
70°C, US 0%	3.79	3.79	3.80	3.80	3.79	0.01
75°C, US 0%	3.99	4.00	4.00	4.00	3.99	0.01
80°C, US 0%	4.13	4.12	4.12	4.12	4.12	0.01
60°C, US 20%	4.11	4.12	4.12	4.12	4.11	0.01
65°C, US 20%	4.13	4.13	4.12	4.13	4.12	0.01
70°C, US 20%	4.10	4.10	4.10	4.10	4.10	0.00
75°C, US 20%	4.08	4.08	4.08	4.08	4.08	0.00
80°C, US 20%	4.07	4.07	4.07	4.07	4.07	0.00
60°C, US 50%	4.09	4.09	4.09	4.09	4.09	0.00
65°C, US 50%	4.06	4.08	4.08	4.08	4.07	0.01
70°C, US 50%	4.09	4.10	4.10	4.10	4.09	0.01
75°C, US 50%	4.09	4.09	4.09	4.09	4.09	0.00
80°C, US 50%	4.11	4.10	4.11	4.11	4.10	0.01
60°C, US 100%	4.09	4.08	4.08	4.08	4.08	0.01
65°C, US 100%	4.07	4.09	4.09	4.09	4.08	0.01
70°C, US 100%	4.07	4.07	4.07	4.07	4.07	0.00
75°C, US 100%	4.10	4.11	4.11	4.11	4.10	0.01
80°C, US 100%	4.10	4.10	4.10	4.10	4.10	0.00

Appendix B

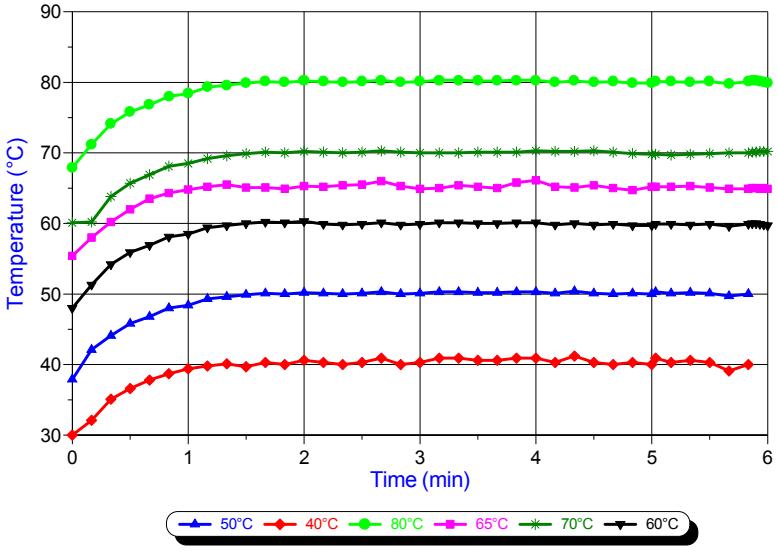
B.1: Temperature profile of treated enzyme at the outlet from the combination treatment of temperature, pressure 100 kPa and sonication power 100% in the lemon PE continuous process



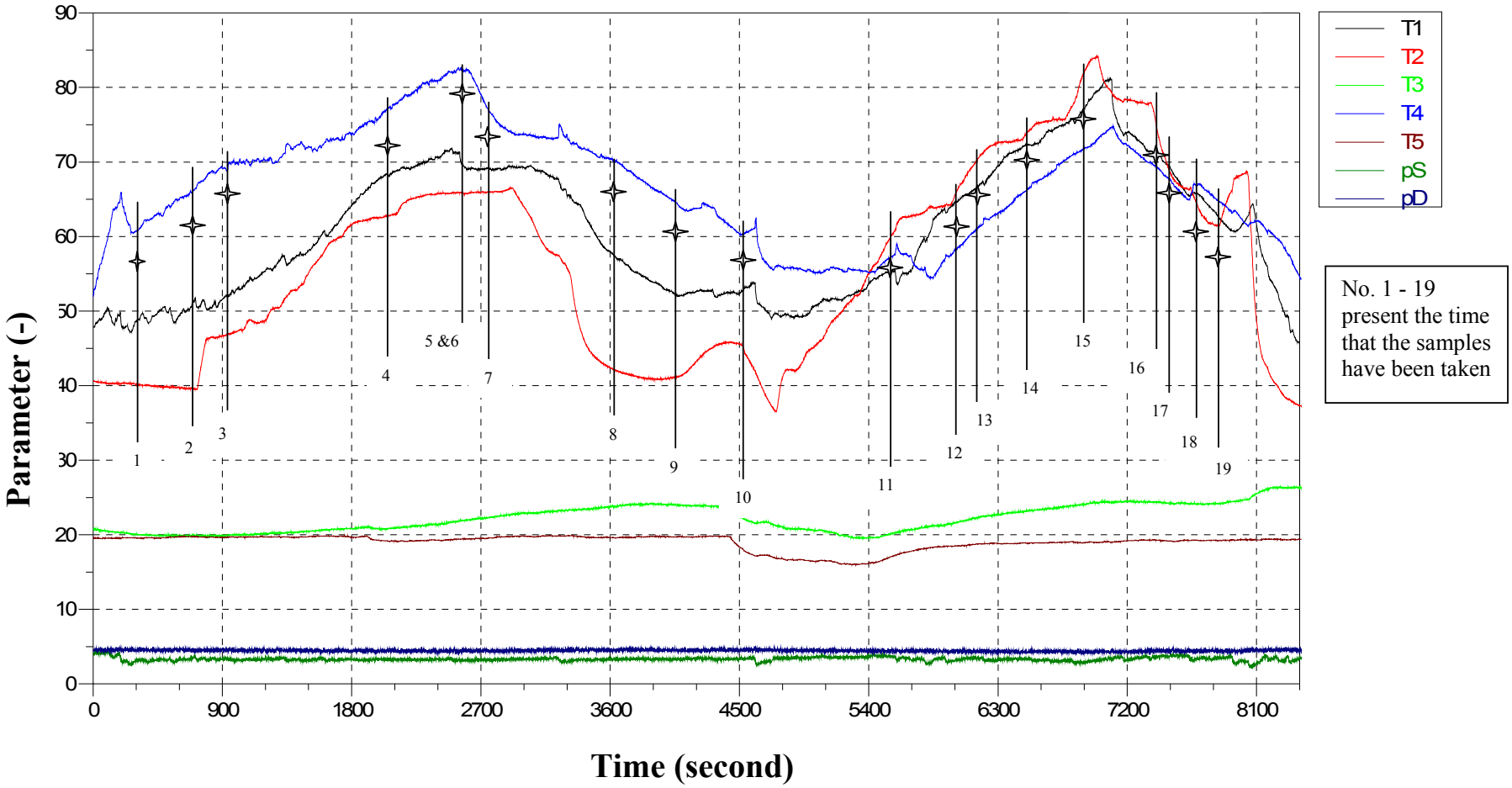
B.2: Temperature profile of treated enzyme at the outlet from the combination treatment of temperature, pressure 200 kPa and sonication power 100% in the lemon PE continuous process

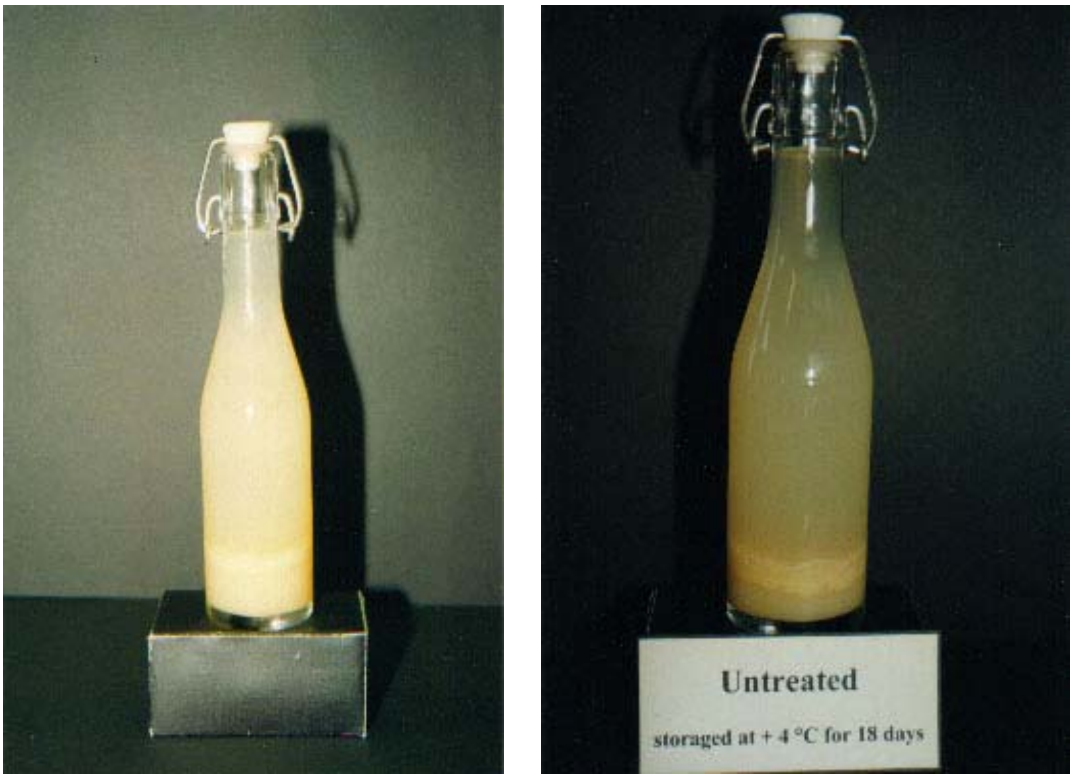


B.3: Temperature profile of treated enzyme at the outlet from the combination treatment of temperature, pressure 300 kPa and sonication power 100% in the lemon PE continuous process

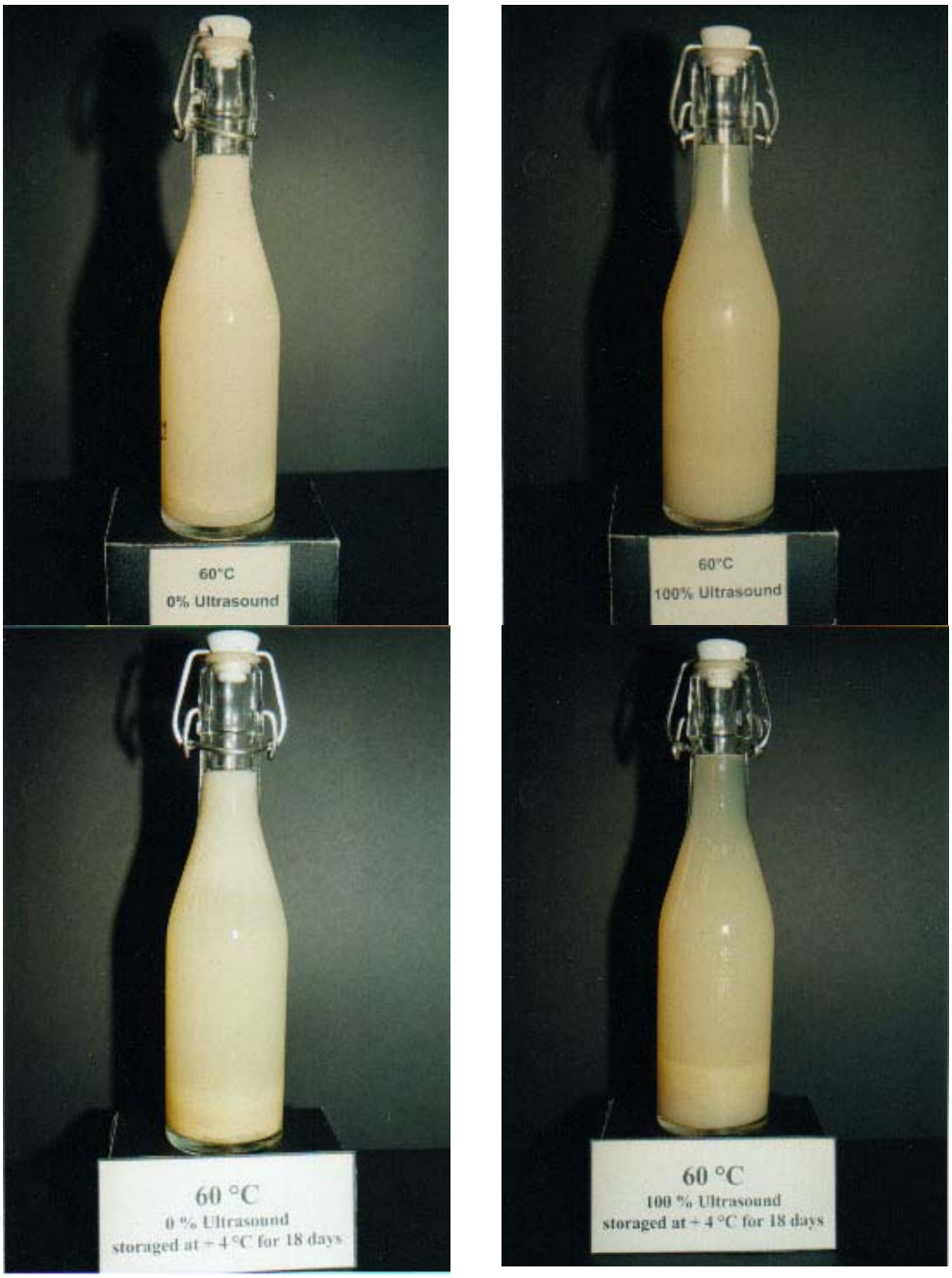


B.4: Temperature profile during the combination process of ultrasound, heat and pressure

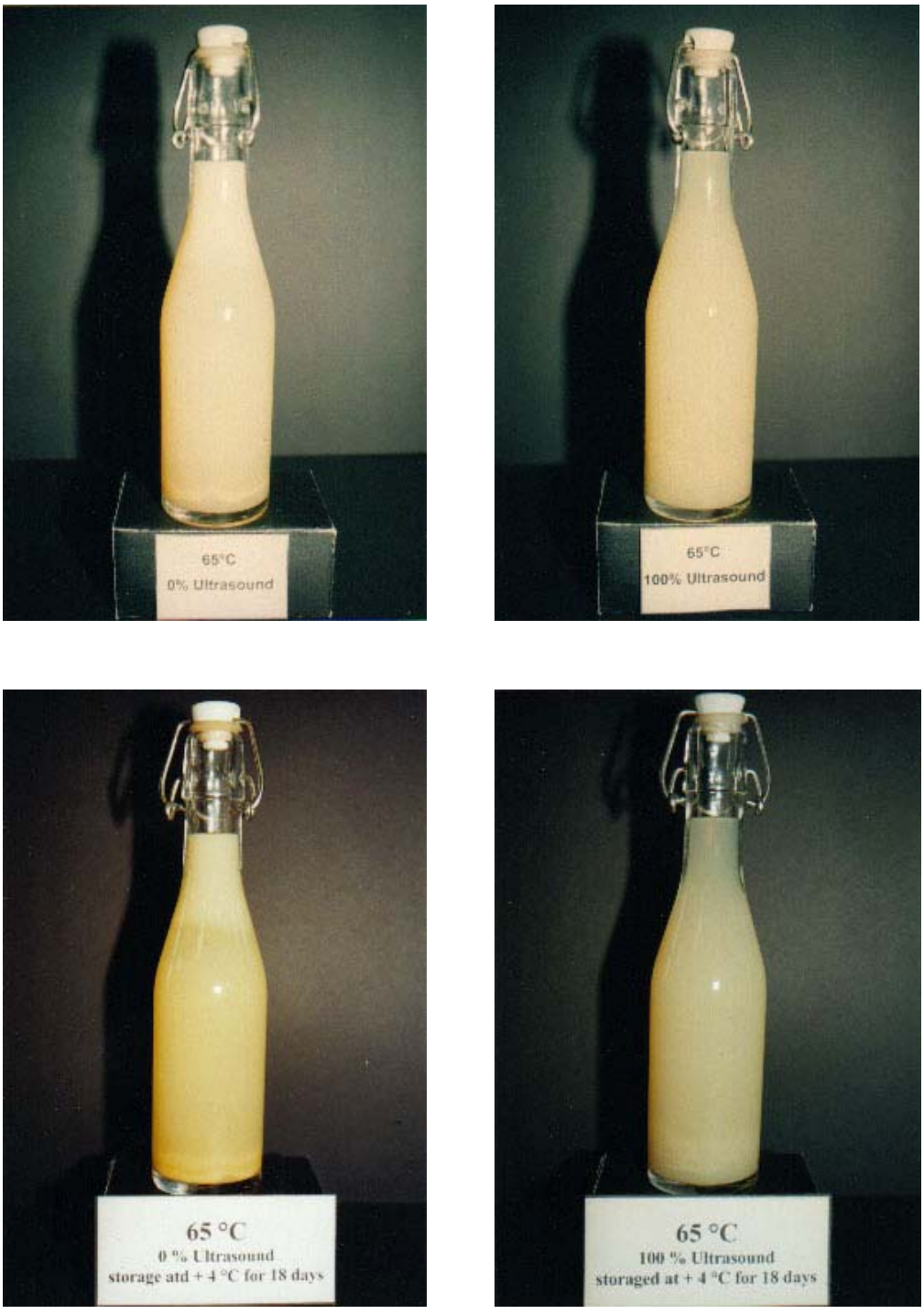




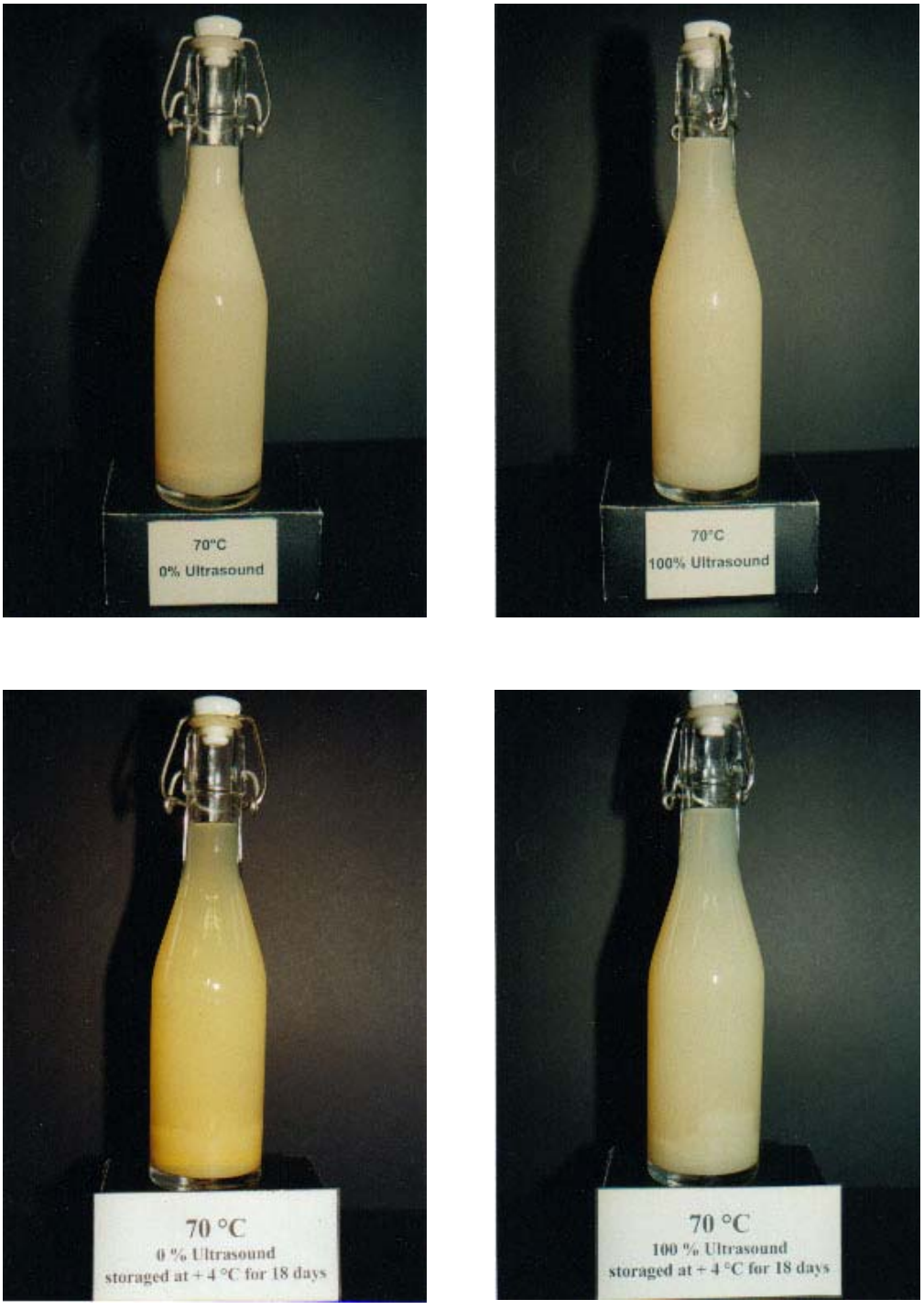
B.5: The untreated lemon juice



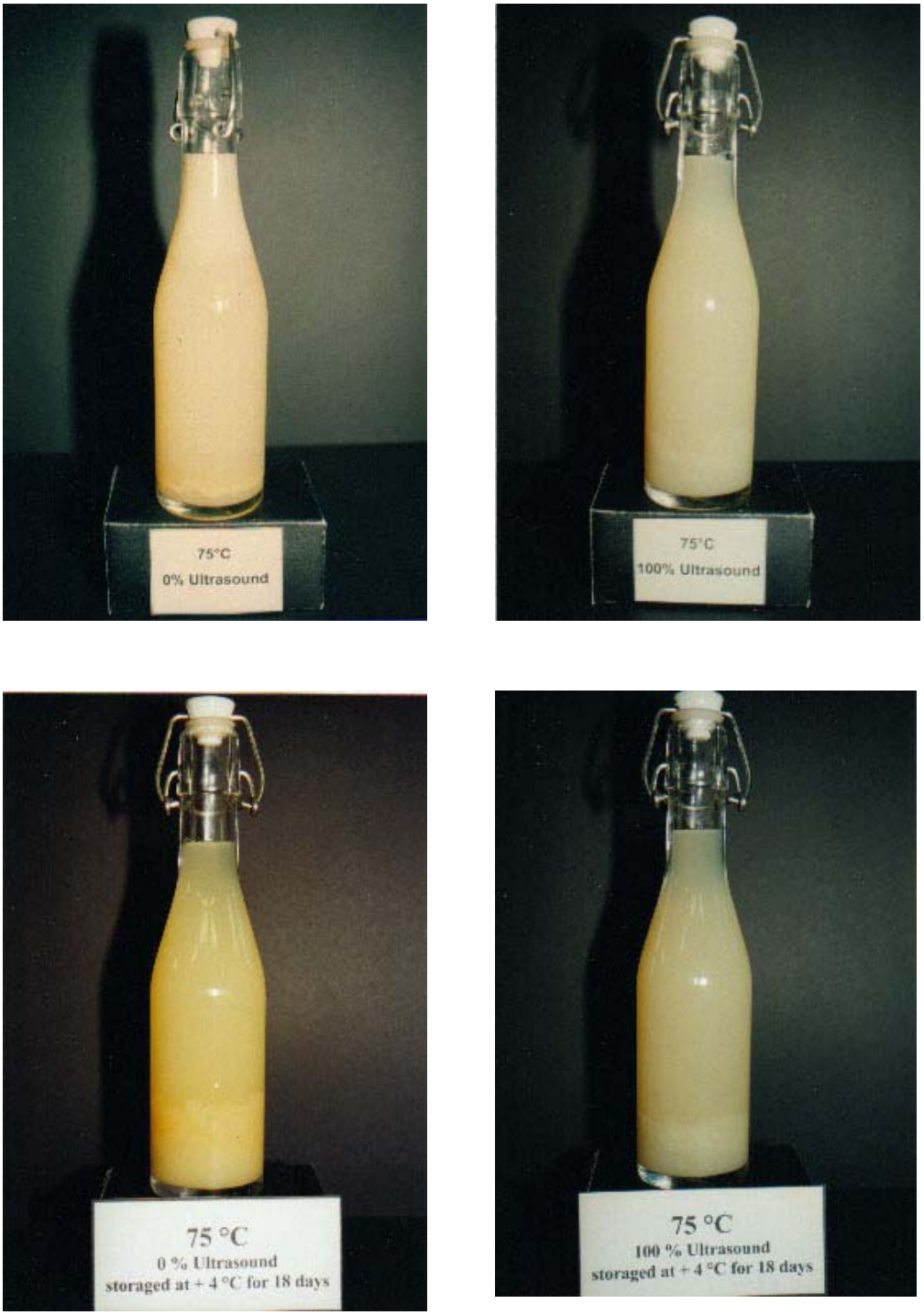
B.6: The treated lemon juice at 60°C, 300 kPa with and without ultrasonic treatment (after the treatment and 18 storage days)



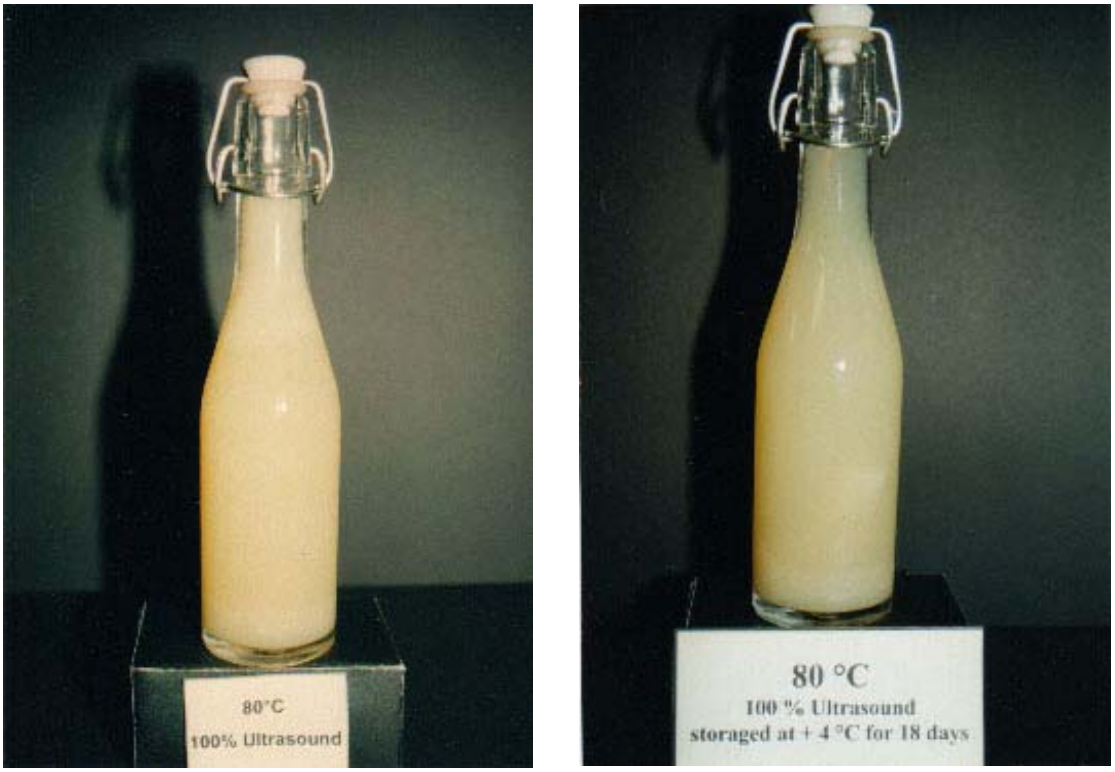
B.7: The treated lemon juice at 70°C, 300 kPa with and without ultrasonic treatment (after the treatment and 18 storage days)



B.8: The treated lemon juice at 70°C, 300 kPa with and without ultrasonic treatment (after the treatment and 18 storage days)



B.9: The treated lemon juice at 75°C, 300 kPa with and without ultrasonic treatment (after the treatment and 18 storage days)



B.10: The treated lemon juice at 80°C, 300 kPa with ultrasonic treatment (after the treatment and 18 storage days)

Part VII

References

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